Final Report

Cholinesterase Monitoring of Pesticide Handlers in Agriculture: 2004

Report to the Washington Department of Labor and Industries

The Scientific Advisory Committee for Cholinesterase Monitoring formed under RCW 49.17.288

MARCH 30, 2005

Executive Summary

The Washington state cholinesterase monitoring rule, Chapter 296-307-148 WAC, was adopted in December 2003 and became effective in February 2004. The rule requires the state Department of Labor and Industries (L&I) to organize a scientific team (the "Scientific Advisory Committee") to oversee collection and analysis of data collected in 2004 and 2005. The rule provides for a review after the first and second monitoring years to gain a greater knowledge and certainty about the extent and causes of overexposure to (organophosphate and N-methyl-carbamate) cholinesterase-inhibiting pesticides. The Scientific Advisory Committee was charged with overseeing collection and analysis of testing data, and making recommendations to L&I and to the cholinesterase monitoring advisory committee. This is the first of its three reports.

Committee members were drawn from academic organizations, state agencies concerned with health and occupational issues, and included experts from cholinesterase monitoring programs in Washington and elsewhere.

Background information on ChE monitoring

Cholinesterase (acetylcholinesterase or AChE) is an enzyme present in many species, from insects to humans. It is required for proper functioning of the nervous system. Tiny gaps called synapses are found between adjacent nerves (neurons), and neurons and target sites (muscles, glands, and organs) of all animals. Nerve signals are transmitted across these synapses by the release of chemicals called neurotransmitters, including. acetylcholine. Acetylcholine regulation is important to proper functioning of the nervous system and muscle contraction, and disruption of this regulation through inhibition of AChE can result in serious nervous system effects.

A number of natural and synthetic chemicals (including some pesticides and medications) can bind to AChE and inhibit its normal activity. Two classes of currently used pesticides, known generically as organophosphate (OP) and N-methyl carbamate (CB) insecticides, are of particular concern to agricultural workers. These workers can be exposed by inhalation, through the skin, by ingestion, and through the mucous membranes and eyes. Monitoring the activity level of AChE in workers can provide an early warning of overexposure, so that steps can be taken to prevent further exposures that could lead to adverse health effects.

The same AChE enzyme present in the nervous system also exists in the membranes of red blood cells (RBC). Another form of the enzyme, butyrylcholinesterase (sometimes called pseudocholinesterase or just cholinesterase), is present in the blood plasma (or serum). Both of these forms of cholinesterase are referred to in this summary using the abbreviation "ChE". The activity level of both of these enzymes can be measured in blood samples. Each pesticide handler is given a baseline blood test prior to handling pesticides and has subsequent blood tests to monitor changes in cholinesterase activity levels. Significant depression from baseline levels indicates overexposure and an increased risk for developing cholinergic poisoning.

.

Overview of the Cholinesterase Monitoring System

Employers identified workers expected to handle[1] toxicity class I and II organophosphate and N-methyl carbamate pesticides for 50 or more hours in any consecutive 30-day period, and selected health care providers to participate in their monitoring program. Each worker was provided training by the employer on the rule and the hazards of cholinesterase-inhibiting pesticides and sent for an initial consultation with the provider. Baseline blood samples were sent to the Public Health Laboratory for two cholinesterase tests: an assay for the red blood cell cholinesterase activity (RBC ChE) and an assay for the serum (plasma) cholinesterase activity (serum ChE). Subsequent periodic tests were given every 30 days, either if the 50-hour exposure threshold was met, or (at the employer's option) on a 30-day schedule throughout the pesticide application season.

If either RBC ChE or serum ChE was depressed below 80% of baseline (that is, more than 20% depression from baseline ChE activity), it triggered follow-up action by the monitoring program. A moderate depression (of up to 30% for RBC or up to 40% for serum ChE) resulted in a review of pesticide handling practices, and a more marked depression beyond these level required a temporary removal from pesticide exposures until the cholinesterase level returned to at least 80% of baseline. Under the rule, health care providers were required to notify employers of the depression alert. A few days after the notification, WISHA's consultation services followed up by contacting the employer to schedule a site visit and interviews with the employer and worker(s).

Lab Analysis and Data Quality

Laboratory methods and practices

Washington decided to use a single laboratory, the Washington State Public Health Laboratory (PHL), to simplify logistical procedures and minimize quality control issues in the first year. This lab is accredited for various clinical analyses, and was fully qualified to perform required ChE measurements. Laboratory procedures used by the PHL were documented in a standard operating procedure ("SOP") formally adopted in August 2004.

The PHL generally followed its stated procedures, although a rush of samples in the first three months of the monitoring season (January - March 2004) combined with instrumental difficulties (lipids clogging a micropipette) created a backlog. About 11% of the RBC baseline samples were held for an extended time, beyond a specified 28-day storage time limit that was subsequently established. It is uncertain whether the samples that were held beyond SOP holding times could have degraded enough to reduce baseline RBC ChE values, leading to underestimation of depressions in periodic tests and thereby

[1] See defintions section 11005 http://www.lni.wa.gov/wisha/rules/agriculture/HTML/part-i-1.htm#WAC296-307-11005

failure in some cases to detect workplace overexposures. There are no indications, however, that serum ChE values were adversely affected. This analysis backlog was corrected in early May, 2004 and did not re-occur. Other aspects of sample and data management procedures specified in the SOP were clearly adhered to, including daily instrument checks and analysis of quality control samples.

Quality Control

The lab, as part of its own quality assurance program, collected quality control data and participated in the CAP proficiency testing program for serum ChE . Additionally, quality control samples were submitted to the lab disguised as actual monitoring samples.

Data variability (precision) was reviewed for different categories of blood samples, and was characterized as being unexceptional and not indicative of poor laboratory practices.

Bias (accuracy) was much harder to assess than was variability (particularly for the RBC assay) because it requires analysis of samples of known ChE activity level. Such materials were not available for the RBC ChE assay, although comparison samples might be available in the future from the University of California-Davis laboratory. Serum ChE values for reference samples indicated that any bias for that assay was small (well under 5%).

A review of procedures used, laboratory quality control results, and actual monitoring findings resulted in several suggestions for method revisions and indicated a need for further method review, particularly for the RBC ChE assay. Different investigators perform this test in different ways, and there is no single authoritative method. In particular, the possibility of analytical drift in the RBC ChE assay requires further investigation of assay performance.

Despite these areas of needed attention, the PH Lab demonstrated good performance on internal and blinded external QC measurements, and reasonable overall assay precision.

Analysis of Cholinesterase Monitoring Results

Statistical analysis

Most analysis of 2004 data was confined to the first periodic test plus accompanying baseline results. The workers' mean age was 36; 99% were male and 93% were Hispanic. Over 2,500 workers had baseline tests, but only 580 had at least one periodic test. Of these 119 or about 20% had drops in RBC and/or serum ChE indicating overexposure to organophosphate or carbamate pesticides.

The population average serum ChE activity decreased significantly between baseline and (first) periodic test measurement. This depression is not explainable by simple biological variability, laboratory error, or changes in laboratory methods. This downward movement is likely the result of pesticide exposure among monitored workers.

In contrast to serum ChE activity, a significant increase from baseline to periodic test in population average RBC ChE activity was found. No clearly identifiable biological or exposure phenomenon easily explains this finding. Further interpretation of RBC ChE as an indicator of pesticide exposures using Year 1 data is not justified, given this observation. The difference in patterns of depression for serum ChE versus RBC ChE may be a function of the pesticide used. For example, chlorpyrifos, an OP insecticide widely used in orchards during tree dormancy, preferentially depresses serum ChE rather than RBC ChE.

Variability in ChE activity from causes other than pesticide exposure is a major influence on the reliability and predictive power of ChE monitoring. In 2004, this variability was measured for serum and RBC ChE as being in the 8-10% range. Applying this observation to actual number of tests run and to apparent occurrence rates for depressions, the maximum number of false positive tests at the 20%, 30% and 40% depression action levels was estimated. At the "exposure removal" level, the reliability of the RBC ChE test was close to 80%, and was significantly greater for serum ChE. A serum ChE value that was 20% depressed from baseline had a greater than 80% probability of being depressed due to an actual change in the activity of the enzyme. For observations of depressions beyond 20%, the reliability increased to greater than 90% for 30% depression and 99+% for greater than 40% depression.

As of September 2004, only about 32% of pesticide handlers on alert or removal status had had L&I site visits. In their interviews, four pesticides repeatedly were mentioned: Sevin (carbaryl), Lorsban (chlorpyrifos), Carzol (formetanate), and Guthion (azinphos-methyl). The greatest proportion of the workers qualifying for alert status had been using only Sevin or Lorsban. The greatest proportion of pesticide handlers classified for exposure removal used a mixture of Sevin and an organophosphorous insecticide (Lorsban or Guthion). Analysis of the relationship between reported hours of pesticide handling and ChE depression was not possible because of limitations in the information available for analysis at the time of this report.

Reducing the number of false positive results by changing regulatory action limits will necessarily increase the expected number of false negatives. Moving the 20% threshold up to 30%, for example, would decrease the number of false positive cases from 6 per hundred to 0.6 per hundred, but would decrease chances of someone with true exposure being correctly classified. The cost of each of these outcomes, true positives vs. false positives and false negatives vs. true negatives must be balanced with awareness of the consequence both for health benefits and for the economic costs of each decision.

Assessment of Program Implementation in 2004

A massive effort went into preparation and implementation of the first season of ChE monitoring. Educational materials were developed, training sessions were held, and a network of providers was formed. Enrollment in the program was much greater than anticipated, leading to the backlog of blood samples discussed earlier. A new information management system for ChE monitoring results ("CMDS") was designed and

implemented. A response system was implemented for cases of apparent ChE depression. For some aspects of the program, measures of effectiveness or quality have yet to be devised.

Timeliness of processing samples and reporting results

Two problems were associated with timeliness. The delays in laboratory analysis of baseline samples that occurred in March and April of 2004 were resolved by mid-May, 2004. In addition, there were delays in reporting baseline results to the health care provider. This seems to have had little observable effect on the providers' delivery of services to the worker, but it could erode confidence in the program as a whole. Better communication would have helped, and the system of reporting results to medical providers needs reconsideration.

WISHA consultation visits

Field investigations following an alert were poorly implemented in 2004. These were designed to ascertain possible causes of overexposure. Thirty-nine employers with at least one worker identified as having 20% or more ChE depression granted permission for follow-up field visits from L&I WISHA consultation staff. As of mid-September, 19 had been visited and reports covering 37 of the 119 employees who had ChE depression had been written. The goal was to make consultations visits within three days of referral (eight days after laboratory testing). The average length of time from referral to consultation visit was 34.5 days.

These site visits identified deficiencies in use of personal protective equipment, equipment cleaning/maintenance, and personal hygiene (mixing/loading/applying and personal protection). It is not possible to assess the effectiveness of field visit information in explaining individual cases of overexposure, or recommended industry-wide changes in work practices. There have been no accepted industrial insurance claims related to the cholinesterase-monitoring program. Only one employee reported experiencing symptoms associated with cholinergic poisoning (transient dizziness and nausea), but he never reported this to his employer. This employee also reported not performing respirator fit checks and said he felt a mist when applying pesticides.

WISHA survey of medical providers

Incidental observations revealed gaps in provider performance, including information missing from lab request forms, inconsistent information on follow-up sample forms that required hand matching of subjects, and slow transmission of test results to employers. There was no clear mechanism for informing workers of their results. L&I expected – but did not explicitly require – that health care providers would notify the workers. There are some indications that medical providers and employers failed to provide proactive evaluation and follow-up in the clinical monitoring program. Examples include failure to retest pesticide handlers who were removed from exposure due to ChE depression, and

failure to report testing results to them. Guidance and oversight of providers during the first monitoring season may have been insufficient.

A survey of medical providers showed a range of attitudes about the program. A small number of responders felt that the program was ineffective, or that it did not address a significant need, while most felt that the program was at least somewhat effective and produced benefits. Comments regarding obstacles to providing monitoring services, follow-up, notification, and training for providers all indicate that the program functioned as intended. However, significant gaps need attention.

Recommendations and Issues

Overall program design

L&I / employers: Reduce the enrollment and baseline testing of workers who do not qualify for subsequent periodic testing.

L&I / WDOH: The system of reporting results to medical providers needs reconsideration to balance the competing needs for rapid recognition of depressions vs. the need to engage providers in case ascertainment and follow-up.

L&I / WDOH: Continue using a single testing methodology and a single laboratory for all individual employee tests to minimize extraneous differences between laboratories.

Program development

L&I/WDOH: Evaluate laboratory resources necessary to process samples in a timely manner.

L&I: An evaluation plan for each aspect of the program (including the roles and responsibilities of employers, providers, workers, and program personnel) should be developed.

Issue: A method to assess the quality of data regarding hours of pesticide use is lacking.

Enrollment

L&I / Growers: identify more precisely workers who are required to participate in the program so that a larger proportion of workers receiving baseline tests also receive periodic tests.

Sample collection

L&I / WDOH: Devise improved sample submission procedures that reduce or eliminate the need for hand matching of samples to workers.

Laboratory analysis

WDOH: Reject samples where integrity or successful adherence to collection and shipping protocols appear questionable.

WDOH: Add hemoglobin determination on each sample, to directly correct for RBC content in the sample and for volumetric error during pipetting of packed red blood cells in the RBC assay.

WDOH: Beyond specific adjustments to current procedures identified in this section, the Public Health Laboratory is encouraged to reassess its overall methods in concert with experts in the field of enzymology, specifically cholinesterase enzyme characterization.

Issue: 2004 RBC monitoring results suggested an unexplainable bias that could lead to under-recognition of enzyme depressions. This observation was applicable even for samples that were not affected by extended holding times.

Issue: An RBC ChE reference material of known stability and certified enzyme activity is needed for assessment of ongoing assay stability and accuracy..

Issue: Routine inter-laboratory comparisons are needed for ChE assays. These should include both baseline and associated periodic samples, to permit comparison between labs of activity differences from baseline to periodic test..

Data analysis and interpretation

L&I / Growers: Improve collection of information describing hours of pesticide handling for each periodic test.

Issue: In a few cases, workers with removal-level RBC depressions showed continuing low ChE activity compared with baseline. It is possible that some of the persistent depressions were due to erroneously elevated baselines or to depression from causes other than workplace pesticide exposures.

Exposure response

L&I: Improve timeliness of WISHA consultation activities.

L&I: Develop and include more quantitative procedures in the standardized checklist that WISHA consultants use for exposure assessment.

Table of Contents

Table of Contents

CHAPTER 1: INTRODUCTION

1.1. Cholinesterase Monitoring Rule Background

In 1993, the Washington Department of Labor & Industries (L&I) adopted a cholinesterase monitoring recommendation for agriculture pesticide handlers. This action was taken in response to agriculture worker advocates' petitions to adopt a mandatory cholinesterase monitoring rule and L&I's recognition of the potential benefits of cholinesterase monitoring. Reasons for not further considering a mandatory cholinesterase-monitoring rule at that time included: 1) lack of an established medical infrastructure (laboratories, medical providers) to administer a statewide cholinesterase monitoring program, 2) lack of an established surveillance system, and 3) the absence of definitive research establishing the appropriateness of utilizing blood cholinesterases as surrogates for nervous system cholinesterase activity.

At the same time that that the cholinesterase recommendation was being adopted, L&I agreed to convene a Technical Advisory Group (TAG) to evaluate and make recommendations on cholinesterase monitoring in Washington State. The TAG released its final report in September 1995. The report reiterated the potential benefits of cholinesterase monitoring but did not advise mandatory cholinesterase monitoring programs. Instead the TAG report recommended retaining the cholinesterase monitoring recommendation already in place and using an interagency work group to identify elements needed and potential obstacles to implementing a useful program.

In 1997 L&I was asked by Evergreen Legal Services on behalf of clients to implement mandatory cholinesterase monitoring. L&I declined to do so, based on consideration of available L&I resources and agency priorities. L&I did not, however, decide that a rule was not warranted. L&I's decision not to pursue rulemaking at the time led to legal action to require L&I to act. In 2002, The Supreme Court of the State of Washington, in *Rios et al v. the Washington State Department of Labor & Industries et al,*[1] upheld the 1993 L&I decision adopting a cholinesterase monitoring recommendation, but also required L&I to initiate rulemaking on a mandatory cholinesterase monitoring rule for agriculture pesticide handlers in response to the 1997 request.

The current cholinesterase monitoring rule, Chapter 296-307-148 WAC, was adopted in December 2003 and became effective in February 2004. As part of the development of the rule, the following measures were taken:

- An advisory group consisting of agriculture worker and grower representatives, other government agencies, and scientific community representatives (the "Stakeholder Advisory Committee") was formed in July 2002.
- Public data-gathering meetings were conducted around the state, including representatives of small businesses that would be affected by the rule.

[1] 145 Wn.2d 483, 39 P.3d 961 (2002)

- L&I also provided a Small Business Economic Impact Statement (SBEIS) and Benefit-Cost Determination (BCD) as required for any rule making.

In order to accomplish a comprehensive review process, the rule requires L&I to organize a scientific team (the "Scientific Advisory Committee") to oversee collection and analysis of data collected during 2004 and 2005. The team was defined as consisting of (at a minimum) representatives from the University of Washington, Washington State University, as well as other interested members of the academic and scientific communities. In addition L&I has organized a stakeholder advisory committee to evaluate rule implementation activities. Table 1.1 lists the membership of the Stakeholder Advisory Committee and the Scientific Advisory Committee, as of October 2004.

Table 1.1: Committee Rosters (* denotes committee consultants)

Scientific Advisory Committee	
Bonauto, David, MD	Associate Med. Director, Dept. of L&I
Das, Rupali, MD, MPH	Public Health Medical Officer CA Dept. of Health Services
Felsot, Allan, PhD	WSU Professor & Extension Spec. Entomology/Environmental Toxicology
Kalman, David, PhD, Chair	UW Chair, Dept. of Environmental & Occupational Health Sciences
Keifer, Matthew, MD, MPH	UW Assoc. Prof. Dept of Medicine & Environmental Health
O'Malley, Michael, MD, MPH	Associate Clinical Prof UC Davis Employee Health Services
Smith, Steven R., MD, MPH	Medical Director, Umatilla Chemical Agent Disposal Facility President, Bi-State Occupational Safety & Health
VanEenwyk, Juliet, PhD	State Epidemiologist for Non-Infectious Conditions WA. State Dept. of Health
*Wilson, Barry, PhD	Professor of Animal Science and Environmental Toxicology UC Davis
* van Belle, Gerald, PhD	Professor of Biostatistics and Environmental and Occupational Health Sciences
Furman, John, PhD, MSN (L&I liaison)	Scientific Committee Liaison, Occupational Nurse Consultant, WISHA, Department of Labor and Industries
Stakeholder Advisory Committee	
Jesernig, Jim	Attorney Represents potato growers
Mayer, Kirk	WA Growers' Clearing House
Nicholson, Erik	United Farm Workers
Tibbetts, Dorothy	Manager, Office of Pesticide Investigation and Surveillance, WA State Dept. of Health
Vega, Griselda	Columbia Legal Services.
Wick, Ann	WA State Dept. of Agriculture Pesticide Management
Keifer, Matthew, MD, MPH	UW Assoc. Prof. Dept of Medicine & Environmental Health
Felsot, Allan, PhD	WSU Professor & Extension Spec.

	Entomology/Environmental Toxicology
Wood, Michael (L&I liaison)	Stakeholder Committee Liaison, WISHA, Department of Labor and Industries

The Washington Department of Health Public Health Laboratory (PHL) was chosen to conduct all testing during the 2004 and 2005 agriculture pesticide application seasons in order to ensure consistency of testing in the absence of an established laboratory testing infrastructure, and to allow efficient collection of surveillance data. Under the current rule, in 2006 testing will be open to commercial laboratories approved by L&I. The PHL may then administer a cholinesterase proficiency-testing program in order to ensure the quality of laboratory services.

Implementation of the rule has included the following milestones:

6/03	Selection of the WDOH PHL to conduct year 1-2 monitoring
9/03	Development of training materials for health care providers
12/03-1/04	Training sessions for health care providers
1/27/04	Beginning of baseline sample collection
3/24/04	Beginning of follow-up sample collection
9/16/04	Cut-off date in CMDS for Year 1 results to be included in year 1 analysis. This includes all samples analyzed as of 9/10/04.
10/1/04	Compiled Year 1 data provided to SAC
11/1/04	(original delivery date) Draft Year 1 review provided to L&I
11/12/04	(actual delivery date) Draft Year 1 review provided to L&I

Charge to the Science Advisory Committee:

This report to L&I is the Science Advisory Committee's initial analysis and recommendations based on 2004 data. The cholinesterase monitoring rule (Chapter 296-307-148 WAC) specifically provides for a review of the experience after the first and second monitoring years in order to gain a greater knowledge and certainty about the extent and effect of overexposure to (organophosphate and N-methyl-carbamate) cholinesterase-inhibiting pesticides. The Scientific Advisory Committee has been charged with overseeing collection and analysis of data, and providing an initial analysis of testing data and any recommendations to L&I and to the cholinesterase monitoring advisory committee by November 1, 2004, and a further analysis and any appropriate recommendations by November 1, 2005. A final report and recommendations will be completed by September 30, 2006. These reports will assist L&I to conduct an objective evaluation of the rule's benefits and to make modifications, or even repeal the rule, as appropriate[2].

[2] The current report was issued in Draft on 10 November, 2004. The Washington Department of Labor and Industries issued its report to the Washington Legislature, "Cholinesterase Monitoring of Pesticide Handlers in Agriculture" in January 2005 as mandated under RCW 49.17.288. That report reference in part information from the draft version of this report and contains additional information about the monitoring program as well.

A primary objective for the Year 1 report is to determine whether any adjustments to the rule are indicated. Implementation issues such as appropriate enrollment of pesticide handlers, timely and appropriate flow of information among employer, worker, heath care provider, laboratory staff, monitoring program staff, and L&I personnel such as field consultants is one example of an aspect of the monitoring program where adjustments might be made. Although the emphasis in this Year 1 report is on patterns in monitoring data indicative of the presence and extent of exposure-related cholinesterase depression, additional issues and concerns are included in Chapter 6 (comments from the Committee) and Chapter 7 (comments from L&I and Stakeholders, with responses from the Committee). A second objective is to evaluate whether the rule has been implemented as intended by L&I.

1.2. Basis for Cholinesterase Medical Monitoring

Cholinesterase (acetylcholinesterase or AChE) is an enzyme present in many species from insects to humans. The action of cholinesterase in the nervous system is to remove the neurotransmitter acetylcholine after release into the synaptic space between nerve cells. The release of acetylcholine and its destruction by cholinesterase occurs almost instantaneously. This system allows for smooth transmission of nerve impulses and termination of that action allowing the nerve cell to respond in a controlled manner. Without adequate levels of active cholinesterase, acetylcholine accumulates in the synapse resulting in over-stimulation and eventual exhaustion of nervous system pathways.

Two classes of pesticides (insecticides), the organophosphates and the N-methyl-carbamates, widely used in production agriculture, are cholinesterase inhibitors. Organophosphate and N-methyl-carbamates bind with cholinesterase preventing cholinesterase from removing acetylcholine from the neuronal synapse. Agriculture pesticide handlers can be overexposed to cholinesterase-inhibiting pesticides when breaks in worker protection protocols occur during activities such as mixing, loading, application, and maintenance of contaminated equipment. Absorption can occur by inhalation, through the skin, by ingestion and through the mucous membranes and eyes.

Significant inhibition in active cholinesterase levels may result in clinical illness (cholinergic poisoning). Overt symptoms are primarily muscle, gland, and organ dysfunctions related to accumulation of acetylcholine in the parasympathetic and sympathetic nervous systems. Symptoms of cholinergic poisoning may include headaches, dizziness, blurred vision, nausea and vomiting, stomach cramps and excessive sweating, constricted pupils, and salivation. Severe poisoning may result in chest tightness, muscle twitching, seizures, coma, and death in rare cases.

Recovery from acute cholinergic poisoning occurs as cholinesterase levels are regenerated through spontaneous reactivation of organophosphate- or N-methyl carbamate-inhibited cholinesterase and through the production of new cholinesterase. The carbamate–cholinesterase bond is weaker than the OP–cholinesterase bond, and therefore spontaneous reactivation of cholinesterase can occur within a matter of hours to

days. The organophosphate-cholinesterase bond may become permanent through an "aging" process; enzyme must then be replaced by new enzyme synthesis. Except in severe cases the treatment for pesticide-related cholinergic poisoning is to simply remove the employee from further exposure until cholinesterase levels regenerate.

There are two types of cholinesterase in blood, plasma (serum) cholinesterase (referred to as "pseudocholinesterase," "PchE," "butylcholinesterase (BuChE)," or "serum cholinesterase") and red blood cell (RBC) cholinesterase (also referred to as "AChE"). In this report, we will refer to these two forms as "serum ChE" and "RBC ChE." These two categories of cholinesterase enzymes found in blood are similar but distinct enzyme groups, with different reactivities and recovery behaviors. RBC cholinesterase is the same cholinesterase (AChE) found in the nervous system and is thought to better reflect effects on the nervous system AChE than does serum cholinesterase.

Organophosphate and N-methyl-cholinesterase pesticides bind with RBC and serum cholinesterase in the same manner that they bind with and inhibit nervous system cholinesterase. Unlike nervous system cholinesterases, blood cholinesterases can be conveniently measured through routine blood collection and laboratory testing methods. The use of the blood enzyme activities as markers for effects delivered to nervous system tissues is based on this similarity in form and reactivity. However, different pesticide products have different binding affinities with either RBC or serum cholinesterase. Monitoring both RBC and serum cholinesterase enzymes provides a more complete clinical picture of exposure.

The following benefits of a cholinesterase medical monitoring program have been identified (Ames, et al 1989[3]):

- Asymptomatic workers with depressed cholinesterase levels can be removed from further exposure thereby preventing possible acute illness;
- Cholinesterase monitoring increases worker awareness of pesticide toxicity;
- Cholinesterase monitoring may prevent possible long-term adverse health effects;
- Cholinesterase monitoring can identify workers with a small but significant cholinesterase depression, triggering a review of work practices to find a source of exposure and prevent further exposure;
- Cholinesterase monitoring can be used in clinical management to prevent re-exposure to cholinesterase-inhibiting pesticides until the worker's cholinesterase activity levels return to his/her normal range.

Organophosphates and N-methyl-carbamates are readily metabolized in the body, producing metabolites that are excreted in urine. Metabolites of organophosphates

[3] Ames RG, Brown, SK, Mengle DC, Kahn E, Stratton JW, and Jackson RJ (1989). Protecting Agricultural Applicatures from Over-Exposure to Cholinesterase-Inhibiting Pesticides: Perspectives from the California Prgramme. Occupational Medcin (39) 85092

include a group of chemicals (ethyl and methyl dithiophosphates), which are common to multiple pesticides, as well as other metabolic breakdown products that can be unique to a specific pesticide (such as paranitrophenol from metabolism of parathion). Urinary metabolites may be eliminated within 48 hours after exposure, requiring that analysis occur soon after exposure if metabolites are to be detected. The presence of specific metabolites in urine indicates pesticide absorption, but gives no indication of physiologic response to exposure. The relative simplicity of blood cholinesterase measurement and its ability show a biochemical response to exposure over time makes a desirable option for occupational cholinesterase monitoring programs.

There are a variety of valid laboratory methods available to measure blood cholinesterases. However, because different assays report results in different units, they cannot be readily compared against each other. Laboratory testing based on the Ellman colorimetric method is the most common commercial testing methodology used today and has been chosen by California, which has had an agricultural biological monitoring program in place since 1974, as its required testing methodology. Use of a single testing methodology and use of a single laboratory for all individual employee tests are desirable in order to minimize extraneous differences within testing data.

Because there are no "universal normal" ranges established for cholinesterase levels and wide inter-individual variation is observed in functional (baseline) levels, it is essential that each individual have baseline blood cholinesterase levels established following a minimum of 30 days from last pesticide exposure and before new exposures to cholinesterase-inhibiting pesticides. Subsequent cholinesterase measurements are then taken on a periodic basis while the employee is handling cholinesterase-inhibiting pesticides. These periodic test measurements are compared to an individual's baseline level in order to monitor exposure. Significant depression in cholinesterase levels compared to the baseline indicates overexposure and an increased risk for developing cholinergic poisoning.

Existing monitoring programs use baseline determinations that are made on the basis of a single test result, or by averaging two samples taken at least 3 days apart. Program guidelines for the California cholinesterase monitoring program indicate that the two baseline samples should show no more than a 15% difference. If the two baseline cholinesterase levels differ by more than 15%, then a third sample is called for, and the two closest tests are averaged. The approach adopted for the current monitoring program is for a single baseline sample to be used.

Proper blood sample handling is important in order to obtain cholinesterase measurements that most closely reflect an individual's cholinesterase activity at the time the sample was obtained. Collection tubes with the proper anticoagulant must be used and mixed appropriately. To limit sample degradation due to hemolysis (red blood cell destruction) the sample should be refrigerated at 4ºC and shipped on ice. Laboratory analysis should be conducted as soon as possible and within 48 hours after sample collection. Samples may be frozen for later analysis for time periods not exceeding established laboratory protocols.

Changes in an individual's cholinesterase levels are determined by calculating the percentage change from baseline. California State, the American Conference of Governmental Industrial Hygienists (ACGIH), and the World Health Organization (WHO), have established depression thresholds. California has established as significant depression thresholds 30% in RBC cholinesterase or 40% in plasma (serum) cholinesterase, respectively. Depressions to these levels require that the employee be removed from exposure to cholinesterase inhibiting pesticides until levels return to within 20% of baseline. A depression of ≥20% from baseline for either blood cholinesterases requires a review of the employee's pesticide handling practices in order to identify and correct any breaches in practice that are contributing to overexposure.

Follow-up testing to monitor recovery from significant cholinesterase depression should be conducted on a schedule determined by the type and extent of depression. RBC cholinesterase is produced along with new red blood cells at about a rate of slightly less than 1% per day. Therefore testing should occur on a schedule based on the percent depression from 80% of baseline. For example, a 35% depression would require that the employee be retested no later than 15 days from the last test. Serum cholinesterase is produced in the liver and regenerates more rapidly than RBC cholinesterase. Testing to monitor serum cholinesterase recovery may be conducted as often as weekly.

CHAPTER 2: OVERVIEW OF THE CHOLINESTERASE MONITORING SYSTEM

2.1. Background

The cholinesterase monitoring system was developed as a result of WAC 296-307-148, the Cholinesterase Monitoring rule, adopted by the Washington State Department of Labor and Industries (L&I). The cholinesterase monitoring system flowchart, **Figure 1** provides an overview of the system including components of the system both required and not required under the rule.

2.2. Enrolling Pesticide Handlers into the Program

In the first year of the rule, employers referred for baseline cholinesterase testing pesticide handlers who were expected to handle toxicity class I or II organophosphate and N-methyl-carbamate pesticides for 50 or more hours in any consecutive 30-day period. Employers selected health care providers to participate in their monitoring program and were required to ensure that the health care provider was familiar with the requirements of the rule. L&I maintained a web page of health care provider resources by county. Published guidelines were developed to assist health care providers understand and effectively administer the cholinesterase monitoring program.[4] Health care providers were to provide information regarding the cholinesterase testing program to the pesticide handlers and offer the option of participating in the testing program. Sample consent and declination forms were provided in the Guideline for Health Care Providers.[1] Additionally, the provider guidelines recommended that the health care provider collect a pre-exposure history and perform a focused physical exam, if indicated by the pre-exposure history.[5].

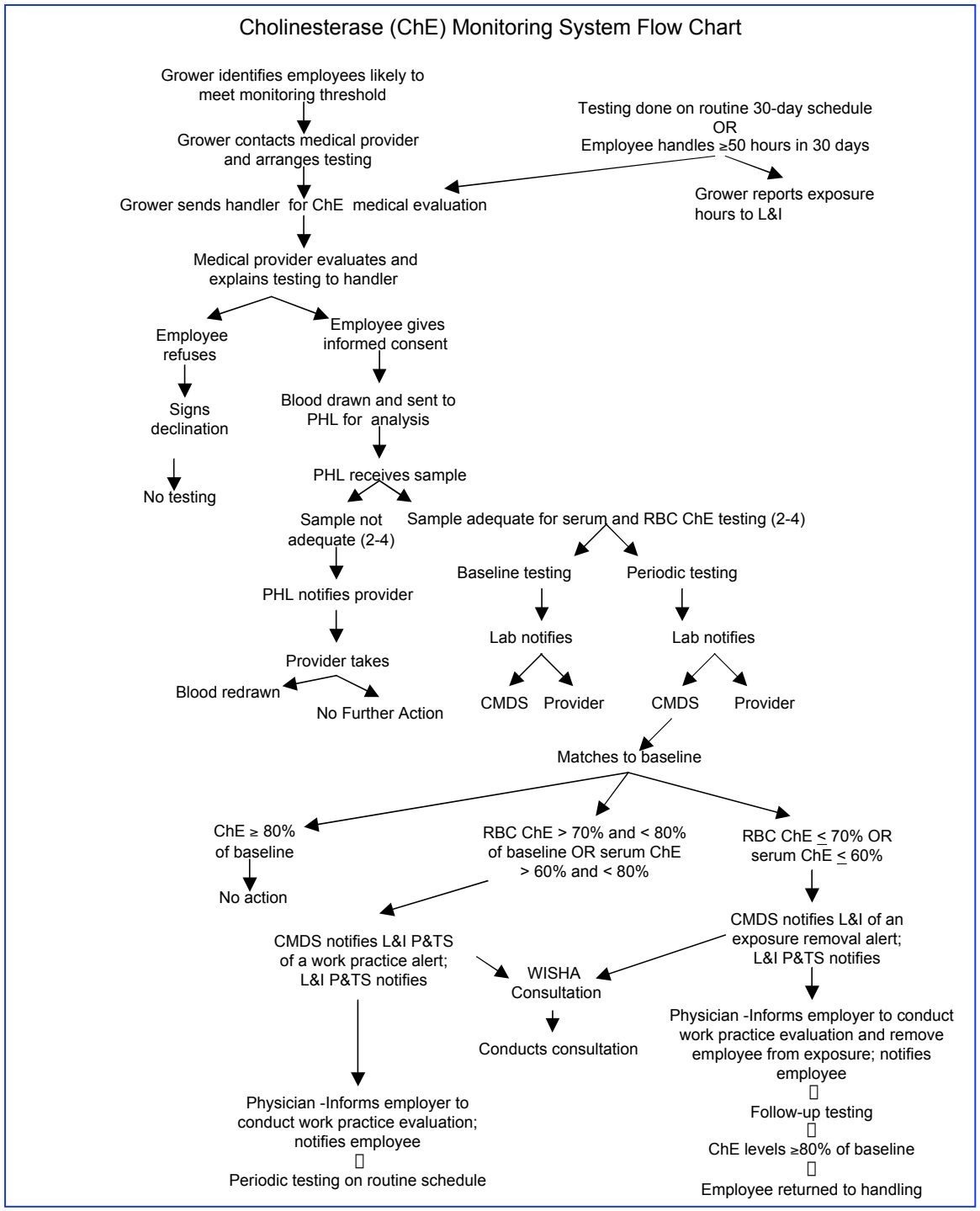
2.3. Collection and Analysis of Blood Sample

If the worker consented to participation in the program, a baseline blood sample was taken. The sample was sent to the Washington State Public Health Laboratory (PHL) which is presently the only laboratory authorized to participate in the cholinesterase monitoring program. The PHL published the Standard Operating Procedure (SOP) for the *Determination of Cholinesterase in Red Blood Cells and Serum by the Ellman Method using Dade Dimension AR Analyzer* which guides collection and handling of the laboratory specimen. The SOP includes parameters for determining if the blood sample is adequate for analysis - including integrity of the specimen tube, appropriate shipping and handling, and completeness of information on the laboratory requisition slip. If the sample was inadequate, the laboratory notified the provider for potential resubmission of another sample. Processing of the blood sample was supposed to be performed within 48 hours of collection in accordance with the SOP. (Compliance with this stipulation is

[4] Boiko P, Keifer M, Furman J, Weyrauch K, Hanks C. Cholinesterase Monitoring for Agricultural Pesticide Handlers; Guidelines for Health Care Providers in Washington State. January 2004.
[5] A sample medical history form is provided on the L&I cholinesterase monitoring web page for medical providers at :http://www.lni.wa.gov/Safety/Topics/AtoZ/Cholinesterase/Providers.asp

discussed in Chapters 3 and 5.) Blood samples were collected for two cholinesterase tests: an assay for the red blood cell cholinesterase activity (RBC ChE) and an assay for the serum (plasma) cholinesterase activity (serum ChE).

Figure 1: ChE monitoring system overview



2.4. Reporting of Results

Upon completion of laboratory testing, PHL reported the results to the medical provider via mail. The results were also transferred electronically from the PHL to the Cholinesterase Monitoring Data System (CMDS) located in the Washington State Department of Health Non-Infectious Conditions Epidemiology program. CMDS was developed to compile test results into an analytic database to monitor and evaluate the program.

2.5. Baseline and Periodic Testing

A baseline test was performed at least 30 days after the employee last handled cholinesterase-inhibiting pesticides. A periodic test occurred every 30 days when the exposure threshold was met or on a scheduled 30-day period through the pesticide application season at the employer's option. The 2004 exposure threshold was 50 or more hours of handling covered pesticides (see definition of "handling" in chapter 296-307-11005 WAC) in any consecutive 30-day period. A baseline test was reported to CMDS with no subsequent action. When a periodic test was performed, the percent depression from baseline was calculated using the following formula:

$$\frac{\text{(Baseline Result - Periodic Result)}}{\text{Baseline Result}} \times 100 = \textit{percent Depression}$$

Providers were instructed to perform this calculation and CMDS also calculated the percent depression. CMDS first needed to match the periodic test to the appropriate baseline. The test records were matched by handler's first, middle and last names, date of birth, place of birth, and mother's surname using probabilistic matching software (Netrics). A new test record was either matched to a pesticide handler already in the database automatically or sent to a file for manual review depending on the quality of the match. In 2004, approximately 65% needed manual review.

2.6. Work Practice and Workplace Removal Alerts

The percent depression, if any, determined the resultant action. If both the RBC ChE and the serum ChE were greater than or equal to 80% of the baseline result, no further action was taken. Employees needed to have additional periodic testing based on subsequent exposure and work intervals.

If the RBC ChE activity was less than 80% but greater than 70% of baseline, or the serum ChE was less than 80% and greater than 60% of the individuals' baseline, a "work practice alert" was generated by CMDS and sent to L&I 's Policy and Technical Services (P&TS). P&TS notified the consultation staff of L&I Washington Industrial Safety and Health Act (WISHA) Services. P&TS also notified the health care provider during 2004, because CMDS often notified L&I of a depression before the provider would have received the mailed results from the PHL. Notification by P&TS minimized time delays that might compromise worker health and safety. The implementation rules required that

the health care provider notify the employer of the need for a work practice evaluation. The work practice evaluation was modeled on the worker protection standard checklist contained in WISHA Regional Directive (WRD) 33.27. The implementation rules for the ChE monitoring system did not explicitly require the health care provider to notify the worker. L&I's expectation, however, was that this notification would occur as part of appropriate clinical practice and the rule required the employer to provide test results to the employee or designated representative upon request. The provider guidelines specified that the health care provider would evaluate the worker to determine if the ChE depression was related to occupational exposure.

If the RBC ChE activity was less than or equal to 70% of baseline or the serum ChE was less than or equal to 60% of baseline, CMDS alerted P&TS of the depression. These levels of depression generated an 'exposure removal alert.' As discussed above in the work practice alert, P&TS notified the health care provider and the WISHA consultation staff. The implementation rules stated that the health care providers notify the employer to remove the worker from pesticide handling and other work pesticide exposures covered by the rule. The provider also notified the employer to conduct a work practice evaluation, as specified above. L&I expected that as part of appropriate clinical practice the health care provider would also notify the worker, schedule follow-up testing to monitor cholinesterase recovery, and evaluate the worker to determine if the cholinesterase depression was related to occupational exposure. The worker was to be removed from exposure until the cholinesterase level returned to greater than or equal to 80% of the baseline level. The medical removal protection benefit contained in chapter 296-307-14830 WAC was in effect until the employee's cholinesterase level recovered or for 3 months whichever came first.

WISHA also offered the employer the opportunity for a consultation visit to evaluate pesticide handling in accordance with WRD 33.27 as part of the response to Work Practice and Workplace Removal Alerts. Every depression alert case was followed up with an offer of field consultation, which was universally accepted by growers.

2.7. WISHA Consultation

P&TS notified WISHA consultation staff approximately five days after receiving an alert from CMDS. The delay of five days was to allow the medical provider enough time to notify the employer and the employer enough time to review workplace practices prior to contact by WISHA consultation services. In addition to providing standard consultation services, the WISHA consultation staff collected surveillance information utilizing a standard series of questions (see WRD 33.27). The questions included worker name, birth date, primary language and number of years as a handler. The employer name was recorded along with additional information regarding the number of acres, crop types, the types of cholinesterase inhibiting pesticides handled, the number of handling hours, employee training, types of pesticide handling activities, use of personal protective equipment (PPE), decontamination facilities, employee symptom history, and identification of the potential cause of exposure.

2.8. Summary of Roles and Responsibilities

The following summarizes the roles and responsibilities of the parties involved in the ChE monitoring system:

Employer (as specified in WAC 296-307, the Guidelines for Health Care Providers, or the PHL's Standard Operating Procedures)

- Identify a health care provider.
- Identify eligible pesticide handlers and provide training as specified in the WAC.
- Send pesticide handlers to provider for initial medical evaluation and exposure-free baseline test.
- Send pesticide handlers to provider for periodic tests and follow-up evaluations.
- Maintain records of handling hours, test results and health care providers' written recommendations.
- Conduct work practice evaluations on notification from health care provider.
    - Make necessary work practice corrections in order to eliminate or minimize the risk of continued over-exposure.
    - Participate in WISHA consultation program (optional).
- Remove employees from handling and other exposures to covered pesticides on notification from provider.
- Follow additional occupational health recommendations from the health care provider.

Health Care Provider (as specified in WAC 296-307, the Guidelines for Health Care Providers, or the PHL Standard Operating Procedures, unless otherwise noted)

- Provide information regarding the ChE testing program to the pesticide handlers.
- Obtain consent or declination for participation in the cholinesterase-testing program.
- Collect a pre-exposure history and if indicated, perform a focused physical exam including pertinent occupational history.
- Collect blood samples, prepare for shipment and ship to PHL.
- Receive test results and calculate percent depression.
- Review differential diagnosis to determine whether a pre-existing condition not related to pesticide exposure, may be causing depression.
- Notify employer of the need for a work practice evaluation or an exposure removal.
- Provide employers with guidance on medical monitoring.
- Notify worker. (This is not specified in any of the documents noted above. L&I's expectation was that this notification would occur as part of appropriate clinical practice.)

Department of Health Public Health Laboratory (first three bullets as specified in the PHL Standard Operating Procedures)

- Assess adequacy of sample upon receipt and notify provider if sample is not adequate.
- Determine levels of serum and RBC ChE following the standard operating procedures.
- Mail results to provider.
- Compile data and transmit electronically to CMDS.

Department of Health Non-Infectious Conditions Epidemiology

- Developed CMDS.
- Maintain CMDS.
  - Manually link follow-ups to baselines that are not matched automatically.
  - Notify L&I of depressions requiring action.

Department of Labor and Industries

- Prepare and distribute provider guidelines.
- Provide training and outreach services.
- Organize scientific and stakeholders advisory committees and review their reports.
  - Publish a list of trained providers and certified laboratories on the Internet.
  - Coordinate recordkeeping requirements with the Department of Agriculture.
- Make efforts to defray costs of medical testing for 2004.
- Conduct rule enforcement and consultation services per RCW 49.17.

2.9. Summary and Conclusions

This chapter provides a summary of the operations of the cholinesterase monitoring system. In general terms, the system seems to have been designed to work efficiently and the Science Advisory Committee does not have recommendations to improve the general design of the system. The Committee was not able to look in depth at how each general process was implemented. Thus, there may be changes that would result in efficiencies for specific processes that the Committee has not identified.

The Committee notes that L&I's notification of providers in the event of work practice and exposure removal alert was not originally planned. Continuing this notification assures that the providers obtain the information in a timely manner, but it obscures the ability to evaluate whether there are time delays if providers receive laboratory reports as part of their routine operations. Thus, the Committee recommends that L&I evaluate the effect of any future changes to this practice.

Ideally, the description of how the system operates will inform the design for future overall evaluation, since each step in the process is subject to evaluation. From this perspective, the Committee notes that there are gaps in the current evaluation. For example, we have not evaluated whether the relationship between the worker and the provider was as L&I expected.

CHAPTER 3: DATA QUALITY

3.1. Overview

This section considers the reliability of monitoring program laboratory results. In the simplest case, a decision would be triggered for one pesticide handler based on only two measured values, the RBC or serum ChE level at baseline and at post-handling follow-up. Analysis of the uncertainty in those two measurements leads to an estimate of the uncertainty of the derived results, "percent depression." Highly uncertain data would still detect and report some of the changes that exceed the current thresholds (20%, 30%, or 40% depression), but a large fraction of the cases detected would be explained by random variation in the measurement rather than by excessive exposure to pesticides. High data variability would also allow a large number of true depressions would go undetected. Conversely, data with very low uncertainty would imply that a much smaller proportion of the detected cases at these depression thresholds were likely the result of random chance, and fewer true depressions would be missed. However, the probability of an individual result being misclassified due to random variation will never go to zero no matter how well the data are obtained.

Two kinds of uncertainty should be evaluated: bias (results that tend to be systematically too high or too low when compared to the true level) and imprecision (results that on average agree with the true value, but scatter above and below it). The ChE assay is a timed kinetic assay and is therefore highly dependent on the detailed conditions under which it is performed. Assay results for a sample divided between laboratories can therefore give results showing poor agreement. However, combining results from sequential measurements done on serial blood samples from an individual (taking a difference or a ratio) will result in the removal of some of this bias, and the percent change from baseline is also more comparable across laboratories. Remaining bias issues include changes in assay response over time (perhaps due to changing batches of reagents, for example).

Accuracy and precision of monitoring data may be affected by several sources of error. Extra-lab sources include:

- Differences in how samples are collected, either between providers or between phlebotomists at a single provider organization
- Variations in sample handling and preparation for shipping: type of container used, anticoagulant used, completeness of centrifugation
- Shipping experience including effects of temperature and degree of agitation

Lab sources include:

- Sample management history including time to sample preparation, duration and temperature of storage prior to analysis
- Assay variability and bias (pipetting of RBC samples is a major source of variability)
- Data management (transcription accuracy, correct calculations)

Even if monitoring data are of perfect accuracy and precision, other (non-pesticide) factors can potentially affect the ChE levels:

- Biological variation (random or of undefined origin)
- Changes due to health status, medications, lifestyle factors, etc. (These factors are expected to affect serum ChE activity more than RBC ChE activity).

This assessment of the 2004 monitoring data quality is based on review of laboratory procedures and practices, and on review of quality control ("QC") data obtained within the lab and from external QC data sets. QC data may be obtained that reflect only within-instrument or within-lab sources of variation, but extra-lab data include both laboratory and non-laboratory influences.

3.2. Laboratory procedures and practices

3.2.1 Methods used

Laboratory procedures have been documented in a standard operating procedure ("SOP") as formally adopted on 8/30/04 that has been used in draft versions since the outset of monitoring activity in January 2004. The SOP was modified in early March, and other aspects of the overall measurement programs have been added since January as well.

Without explicitly reviewing the overall background and current status of the Department of Health laboratory, it is worth noting that this lab has a number of accreditations for various clinical analyses, and appears to be fully qualified to perform these measurements in terms of staff and overall laboratory organization.

The SOP addresses the major aspects of laboratory quality control and includes appropriate control procedures such as daily verification of instrument performance, multiple QC samples included with each batch of field samples, and data review and validation.

Review of the procedures as written has identified several issues for further discussion with the lab and for possible follow-up or corrective action. Most of these issues are related to the biochemical basis of the tests as a true reflection of the status of ChE enzymes in nervous system tissues. The selection of blood cholinesterase itself is a compromise that may fail to perfectly reflect nervous system AChE status; in this regard, the RBC assay is more closely related to target tissue than is the serum analysis, because the acetylcholinesterase enzyme in RBCs is considered to be the same as in nervous system tissues. Additional approximations that underlie the current method and that could affect the predictive power of assay results include:

1. The use of a single enzyme substrate, acetylthiocholine, to assess both RBC ChE activity and serum cholinesterase activity. This substrate, although more convenient in that it allows both assays to use the same test reagents, is not the

optimum choice for the serum assay, and will give nonlinear responses and higher detection limits for low activity levels.

2. The use of an automated "turn-key" instrumental analyzer, without demonstration that measurements are made in the linear response range of each enzyme and under optimized pH and other concentration conditions. The interval between instrumental readings used to determine a rate of change may be too small to accurately detect all activities within the range of interest; the laboratory at this point relies on the instrument manufacturer in this regard.
3. The current method for conversion of activity to a hemoglobin basis assumes constant hemoglobin for a given quantity of packed red blood cells. This assumption should be demonstrated to be true. Assuming this conversion factor rather than measuring hemoglobin will lose an opportunity to correct for volumetric error during pipetting of packed red blood cells, a major contributor to RBC assay imprecision.

Other issues that might result in changes in SOP include the exclusion of samples with evident hemolysis (disruption of red cells) affecting serum and the use of hemolyzed but not solubilized RBC samples for analysis. Since the RBC enzyme is bound to cell membranes, dissolving those membranes using surfactant results in better sample homogeneity and greater assay precision. None of these issues clearly indicate major or known flaws in the data collected according to data analyses performed thus far, but do represent possible ways of reducing variability or bias.

3.2.2. Lab practices

The initial period of monitoring was one in which the lab was unable to produce results it deemed reliable, due to a combination of unexpected numbers of samples that exceeded its capacity, and the need for method troubleshooting and development indicated by the experience with the first samples received. From the outset of the program in January through most of March, only an initial set of 560 samples was analyzed and none were reported, while some 2147 baseline samples were received. Beginning with the week of March 28, the lab analysis was routinized and the backlog of samples awaiting assay was steadily reduced, until it was completely eliminated by early May. The reporting of results also followed routinely, with elimination of the reporting backlog being accomplished by mid June. Through the week of August 29, some 3856 samples were received and reported. Possible effects on data quality of this backlog are discussed later in this chapter.

Review of the lab's practices during the 2004 season (January through mid-September) was accomplished by examining overall records related to sample receipt, within-lab QC measures, and review of full documentation for a random batch of samples.

3.2.3. Data completeness

Completeness is very close to 100%.

3.2.4. Sample receipt and integrity

Overall, 27 samples were rejected, a shipping failure rate of 0.7% (=27/3856). Lab records indicated good adherence to temperature verification procedures (at receipt, the temperature must be ≤10°C; the actual temperature was noted on each sample submission sheet examined). Discussion with lab staff indicated some variation in provider performance with regard to containers used deviating from the ones specified, and submission of some inadequately centrifuged samples. In general, the staff felt that SOP requirements with regard to temperature were very clear and that non-conforming shipments were readily identified and rejected. Overall, sample integrity in shipping appeared to be adequate for the purposes of this program. The sections in the SOP that specify procedures to be used when rejecting a shipment appear to have been followed.

3.2.5 ***Adherence to assay protocol***

In general, the SOP requirements for receipt of samples were adhered to (circumstances permitting). Acceptance of questionable samples was perhaps the area where the SOP was least rigorously applied. For example, hemolysis is noted in the SOP as a sample rejection criterion, but spot checks showed samples (example: lab # 3207) where hemolysis was noted but sample was accepted.

As discussed above, the biggest departure from SOP requirements was the extended holding times for some baseline samples. These results were included in the monitoring database, and are apparently not flagged. Since the database allows one to calculate holding times, it is possible to still consider these results separately from those samples analyzed within SOP holding time limits.

Handwritten records indicating daily instrument performance checks and handwritten annotations on test result printouts from the autoanalyzer indicated that the lab followed SOP requirements. Daily QC results include (beyond instrument performance tests) control samples, blanks, and 1-in-10 duplicate preparations of randomly chosen field samples. These practices provide data that are useful in characterizing the in-laboratory component of measurement error. Specious results due to instrument malfunction would have to survive daily instrument performance tests, comparison of duplicate instrument readings as performed on every sample, and an assay range test. Examples of questionable results that had been flagged and corrective actions taken were provided.

3.2.6. Sample holding times[6]

[6] This discussion is complicated by the fact that SOP requirements were not entirely in place at the start of the monitoring season. The first draft SOP was available in January of 2004. The 28-day holding time limit was not included in that version. The SOP that was finalized in August of 2004 stated that samples could be stored for up to 4 weeks (28 days) at -20 degrees but for longer storage times a freezer temperature of -70 degrees should be used. This was a "post facto" addition to the SOP because of observations made in the laboratory relevant to sample re-assay values. Guidelines regarding storage conditions and holding times were updated in January 2005, based on additional stability study results.

Two criteria are specified in the SOP: the samples should be prepared for freezer storage within 48 hours of collection, and RBC samples must be stored at –20°C and analyzed within 4 weeks. All samples met the 48 hour time limit for processing. Only RBC samples from the Jan-March submissions (all were baseline samples) exceeded the second criterion. Of the 2655 baseline monitoring samples reported to L&I, 432 exceed the SOP holding time limit of 28 days. The laboratory has since conducted additional studies to assess sample stability at –20° C; preliminary results suggest that samples stored at –20° C compared with –70° C show no differences for at least 6 weeks. Of the baseline samples, 375 were held longer than 42 days. The maximum was up to 100 days storage. Considering the total number of cases with both a baseline and a follow-up, 93 of 611 cases exceeded 28 days storage, and 80 of 611 exceeded 42 days. Reassay was conducted on 3 sets of samples: baseline and follow-up samples that showed unusual degree of change (depression), and a random set of samples. These results, shown in Figure 3.1, indicated good agreement between original and repeat test results for serum cholinesterase levels, but an apparent shift to lower levels for RBC, especially in the baseline samples. An explanation for this could be the loss of enzyme activity upon storage, leading to lower results on retest. In Figure 3.1, the results for original and repeat analysis of sample from different groups (baseline, periodic tests) are plotted. If the second result was identical to the first, the resulting data point would fall exactly on the line denoted "perfect agreement." Scatter away from this line indicates results that differ; scatter below the line indicates that repeat test results were lower than the original results. Scatter outside of the lines marked "+ 20%" and "–20%" indicates values that differed between initial and repeat tests by 20% or more. Note the different behavior for RBC versus serum ChE.

Figure 3.1: Repeat analysis, RBC and serum (plasma) ChE

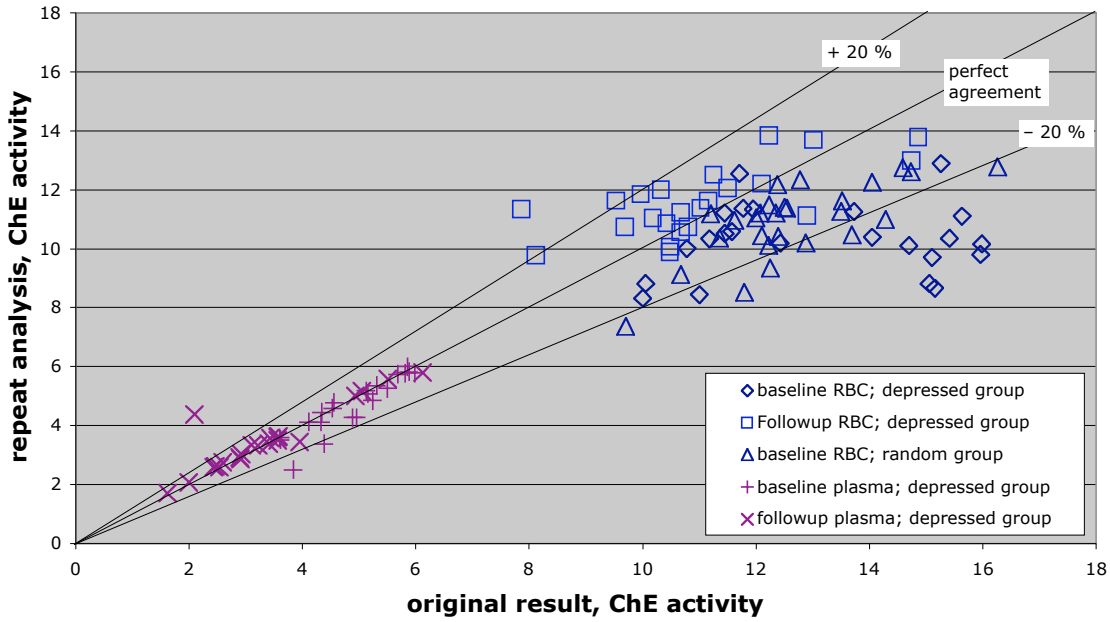


Figure 3.2: RBC ChE retesting vs freezer time

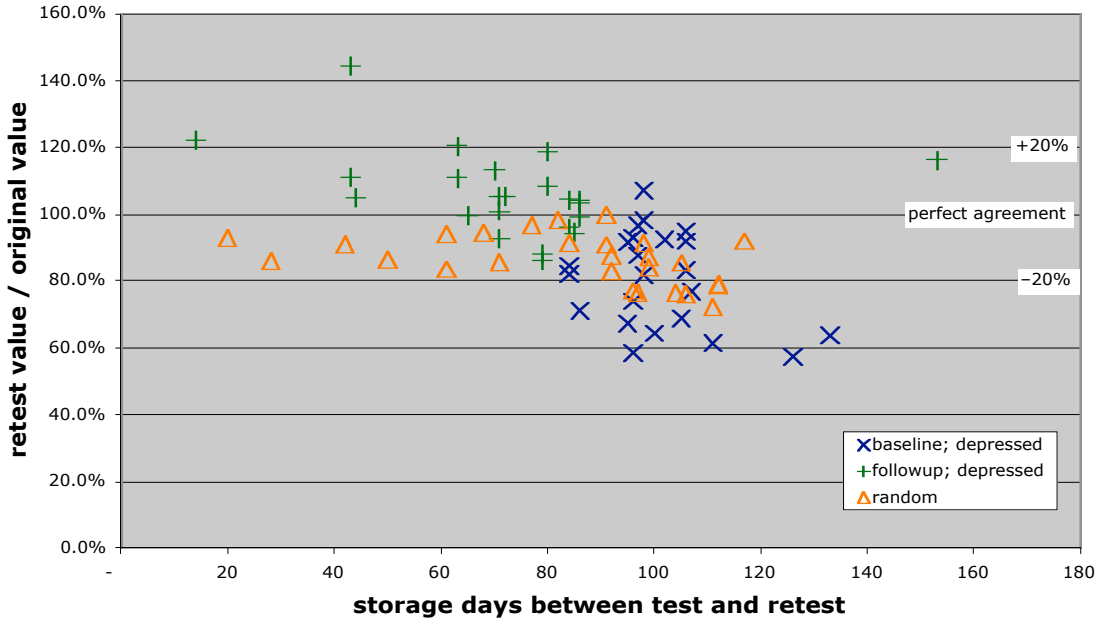


Figure 3.2 presents a scatter plot of the ratio of repeat to original results as a function of storage time. In this case, the value plotted is {result 2/result 1}; perfect agreement is the "100%" line. The data are arranged according to elapsed time between test and retest.

Crosses and "x" symbols denote samples selected for reanalysis because of high depressions seen when initial baseline and periodic samples were compared. Triangles represent reanalysis of a group of baseline samples selected at random. There is a tendency to see lower recoveries upon retest (<100% on the y-scale) for samples held longer periods (especially for crosses and "x" symbols – little time trend is evident for triangles). However, a similar plot using assay date rather than storage days reveals a related pattern, because the samples that had been assayed earlier in the season had longer between-test holding times.

At this point it is not possible to tell if this is a holding time effect or an assay date effect, but in either case, it appears to be uniquely related to the initial start-up of monitoring, and it appears to apply to the RBC assay but not the serum assay.[7]

The possibility of loss of activity in storage exists and would be expected to produce fewer apparent depressions than actual. Apart from loss of RBC activity, there is a suggestion of increased variability in samples stored for extended periods, which could produce both false elevations and false depressions.

[7] Some ChE depression alerts called by the monitoring program were subsequently rescinded based on retesting of stored baseline values. A discussion of these cases and of the Monitoring program's approach to the issue of sample holding times for RBC analysis is contained in the January 2005 report to the legislature cited in footnote 2.

Examination of actual monitoring data (RBC ChE activities versus sample holding times) does not indicate a large increase in variability or a large decrease in RBC activity based on holding times; however, the large person-to-person variation in RBC values could mask such an effect if it were smaller. Examination of a scatter plot of changes in RBC ChE according to baseline sample holding time shows no clear trend, although there is a suggestion of a bias toward elevations at long holding times (which would be consistent with loss of baseline activity). For our analyses of year 1 results, we have not excluded any data based on holding times.

3.3. Evaluation of Year 1 QC data

Year 1 quality control data consist of: (1) routine laboratory quality control results, (2) "special" data sets from QC experiments run by the lab, and (3) external quality control data produced by submission of blinded samples. An additional measure of laboratory data quality that was not implemented in Year 1 was splitting of samples or control materials with other laboratories, or with a single "reference" lab. The specific data sets considered are:

1.a. instrument performance test data, obtained as part of each day's analysis routine.

1.b. Laboratory control materials included in each batch of unknown samples. These are materials that are treated as actual samples but are available in many identical portions and generally have known or certified values for ChE activity.

1.c. Duplicate portions of actual samples assayed as part of each batch of samples, performed randomly on every tenth sample.

2.a. Repeat analysis experiments (both samples showing high depression and a random selection of samples).

2.b Repeat analysis of pooled RBC samples to assess storage stability.

3.a Blind duplicate blood samples submitted by individuals, organized by the Washington Farm Bureau (plus some subsequent periodic samples).

3.b Blind duplicate blood samples submitted by the Washington Department of Labor and Industries (plus some subsequent periodic samples and duplicate periodic samples).

In addition, actual monitoring samples were analyzed with respect to sources of variance (detailed in Chapter 4), which yielded additional information about data quality.

3.3.1. Data precision

Data precision is as indicated by these measures is summarized in Table 3.1. This table expresses data variability as "% CV" (percent coefficient of variation, calculated as the standard deviation divided by the average for a data set). This allows easier comparison of variation for data sets with different average values.

In general, variation is very small for serial readings taken from a single sample in the instrument, and somewhat larger but still small (typically less than 5% variation) for duplicate samples prepared on the same day and run in the same analytical batch. This was equally true for lab duplicates and for blind duplicates disguised as actual samples from different subjects. Somewhat tighter precision was seen for serum compared to RBC ChE results. Repeated samples assayed on many different days was less precise yet: repeated control samples showed 6.4% and 4% CV for RBC and serum ChE respectively, and actual repeated study samples (selected in part from extreme values for depression, so this may be a worst-case comparison) showed 12.6% and 8.3% CV for RBC and serum ChE.

Table 3.1: QC summary		estimated %CV	
Data considered	Sources of variation included	RBC	Serum
1. Duplicate measurements	Instrumental precision only	1.3%	0.5%
1.c. Lab duplicates	Within-batch assay precision	4.9%	**8.3%
1.b. QC control samples	Assay precision over time	6.4%	~ 4 %
3a. Blind field replicates	Within-batch assay precision[8]	6.5%	1.6%
2a. Repeated samples	Assay precision over time + storage effects	12.6%	8.3%
*Monitoring results	Assay+sampling precision + within-person variation	8.8%	9.5%

* These findings are discussed in Section 4 of this report.; ** exclusion of 1 of 27 pairs of lab duplicates reduces this %CV to 1.1%

The finding from actual study data (Chapter 4) that non-exposure sources of variability (including sampling, analysis, and within-person biological variability) were 8.8% and 9.5% CV for RBC and serum ChE, respectively, is generally consistent with the laboratory contribution to variation noted here. This level of variation in laboratory results is not exceptional for these assays and suggests good laboratory performance. The quality of the data overall are reasonable in terms of precision, although samples with prolonged storage and/or early data sets are likely to be less repeatable than this overall average. As is described in Chapter 4, improvement in assay precision or reduction of other contributions to variability in monitoring data would benefit the predictive power of these results.

3.3.2. Accuracy

Accuracy is much harder to assess for these data than is variability, particularly for the RBC assay. In the absence of a known correct value for samples that can be compared with measurements, two approaches that can be used are the measurement of reference materials having known values for ChE activity, and comparison of laboratory results with a pool of analyses from other labs. In the case of RBC ChE there is no certified

[8] Overall variation among 53 pairs of replicated samples expressed as a coefficient of variation is calculated as shown in Appendix 1

reference material that has been available for use. There is some prospect for obtaining more comparison data by use of control materials provided by Dr. Wilson's lab at UC Davis, but these will need to be prepared specifically for this purpose. There are limited opportunities for inter-lab comparison. Further, because of the nature of the assay, the comparability of individual sample results between labs is not a good indicator of the accuracy of within-lab changes in ChE activity seen within individuals. Interlab ("CAP") proficiency testing, which has been used successfully by the PH lab, is directly applicable to serum ChE but not to RBC samples.

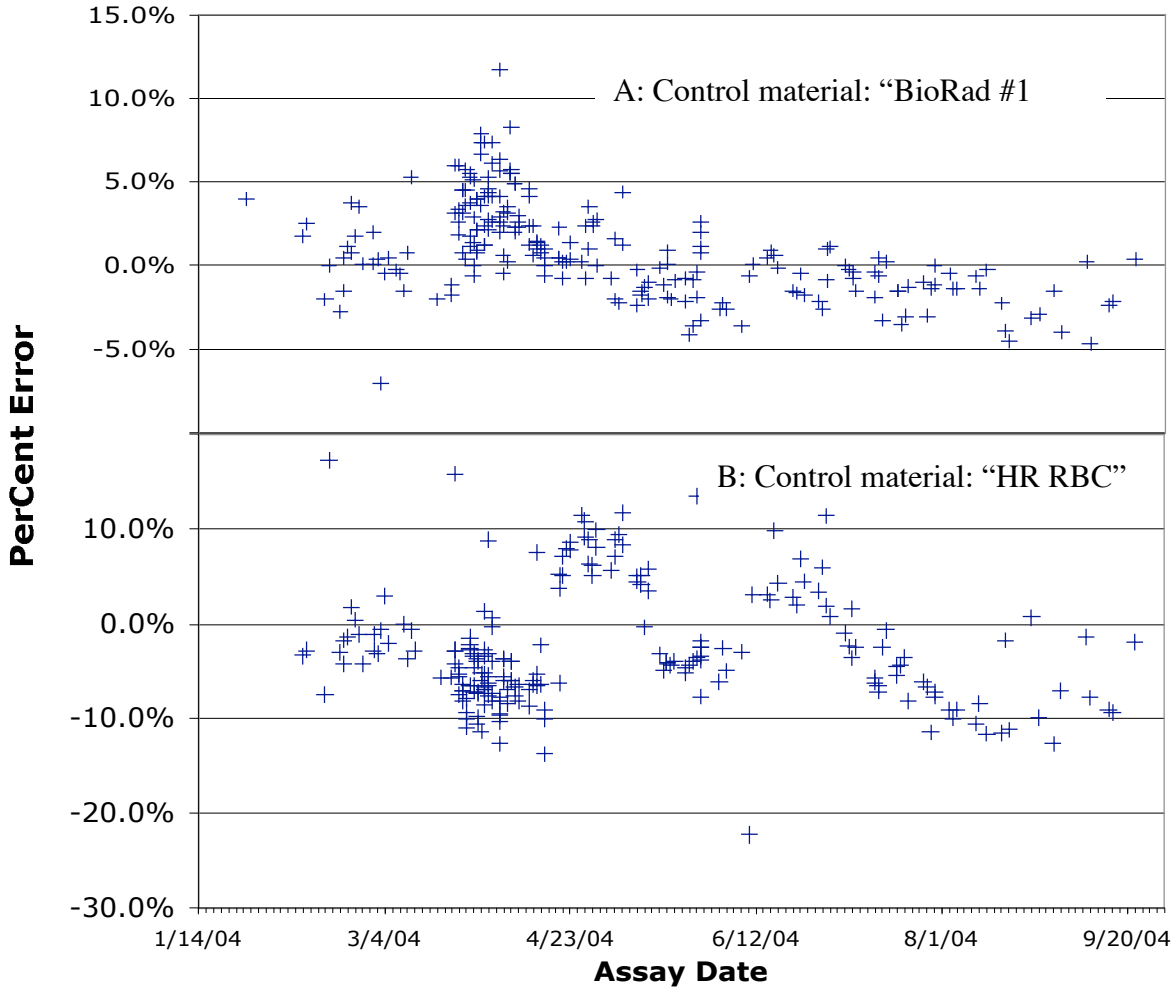


Figure 3.3 – Quality control results for serum and RBC ChE assays. "Biorad #1" is a commercial plasma ChE source that was tested periodically and the measured levels compared with manufacturer's reference value. The "HR RBC" material was prepared by the PH lab, with an average result serving as a reference value. The PH lab performance is expressed as the percent error (above or below) the reference value.

The lab did perform regular QC tests on its serum cholinesterase assay by using commercial control materials. These results would be most applicable to serum ChE assay results, and are summarized in Figure 3.3 and Table 3.2. Figure 3.3 presents a scatter plot of results from control samples that are assayed daily with each batch of field

samples over time; Table 3.2 summarizes the overall results for these samples. The comparison of the measured values with reference values as provided by the manufacturer allows a "percent error" to be calculated (defined as: {100 x (measured value – reference value) / reference value}). The materials "BioRad 1, 2, and 3" are fortified human plasma materials with cholinesterase levels and ranges provided. "Precinorm U plus" is a control material with about 70% of the activity from serum cholinesterase and 30% from RBC cholinesterase. The material labeled "HR RBC" in Table 3.2 and Figure 3.3 is a pooled hemolyzed RBC sample from lab staff, frozen in aliquots. There is no reference value, but a long term average value has been established.

Low values for mean error for each control material in Table 3.2 indicates little overall bias: zero bias would be indicated by a mean or average error of zero (as many high values as low values).

These results support the following observations: (1) there is no long-term trend in these results; (2) RBC results are more variable than serum results; (3) Bias (as indicated by whether the "% error" data had an average value close to zero) was insignificant for all samples but BioRad #3.

		RBC			plasma		
data	notes	N	% CV	mean error	N	% CV	mean error
lab control samples	(N = assays)						
BioRad #1					224	2.8%	0.07%
BioRad #2					208	2.5%	1.45%
BioRad #3					177	7.8%	9.4%
Precinorm		220	3.2%	4.1%	220	3.2%	4.1%
HR RBC	no reference value	216	6.4%	-2.8%**			

** average deviation from expected value established by the lab

Table 3.2 – Results for control samples run in each assay batch

In summary, standard measures of lab performance, especially repeatability measures, indicate reasonable performance for these assays. Although some baseline analyses did not conform to SOP requirements in terms of holding times, attempts to detect a time trend in either ChE activities or in changes in activity between baseline and follow-up did not disclose any detectable trend. Likewise, holding time for baseline samples and number of samples per day were examined and found to have no effect on results. The lack of a reference value for a RBC benchmark sample and resulting inability to characterize method bias was noted. Because the monitoring result that will indicate exposure status is the ratio of two measured values, a constant (proportional) bias in ChE activity per sample will not affect the outcome of this calculation.

None of these factors provides a measurement-based reason for the overall increase in RBC ChE activity between baseline and (first) follow-up noted in Chapter 4. This trend is evidence for either: (1) net decrease in exposure between baseline and first follow-up for the workers as a group, or (2) an artifact affecting RBC ChE activities for this whole group having a net trend toward higher activity for follow-up; or (3) a measurement or sampling artifact that leads to bias in the difference between baseline and follow-up. While effect (1) or (2) could conceivably happen in individual cases, a population-wide trend is very unlikely.

To investigate possibility (3), control sample results shown in Figure 3.3 were used. While season-long average results for control samples shown in Table 3.2 are generally small, for a given control material there are shorter time periods where bias is evident (note clusters of values higher or lower than 0.0 percent error for some time periods in Figure 3.3). A second point is that baseline and follow-up samples are not distributed randomly, but fall into specific time windows. To explore the possibility that actual results were affected by the combination of periods of high or low results coinciding with periods of predominantly baseline or predominantly periodic samples being tested, the following analysis was performed: for every actual RBC measurement, the RBC control material (HR RBC) result for that date (or the daily average for days with multiple HR RBC results) was identified. For every pair of baseline and periodic measurements, the corresponding pair of HR RBC results was converted to a "% depression" value. (These values are really repeat observations of the same material, but at non-random times matched to monitoring samples). The population of these synthetic "depression" results that corresponds to the baseline/first follow-up for the whole monitoring program was examined and is depicted in Figure 3.4. The average result for pairs of HR RBC measurements was +6.3% change (elevation) for these dates, compared with the overall . average bias of – 2.8% obtained across all HR RBC values shown in Table 3.2.

The scatter plot shows the "synthetic" or control RBC ChE change versus that seen for actual monitoring samples analyzed during the same time interval. The following features are significant: (1) there is no correlation between control sample results and actual results – this is not a batch-to-batch systematic variation that could be corrected; (2) both data sets show an excess of elevations over depressions; (3) the control samples show this effect to a greater degree than the actual samples (6.3% vs. 1.5% average change from baseline to follow-up).

Figure 3.4: **RBC ChE change: synthetic vs real data**

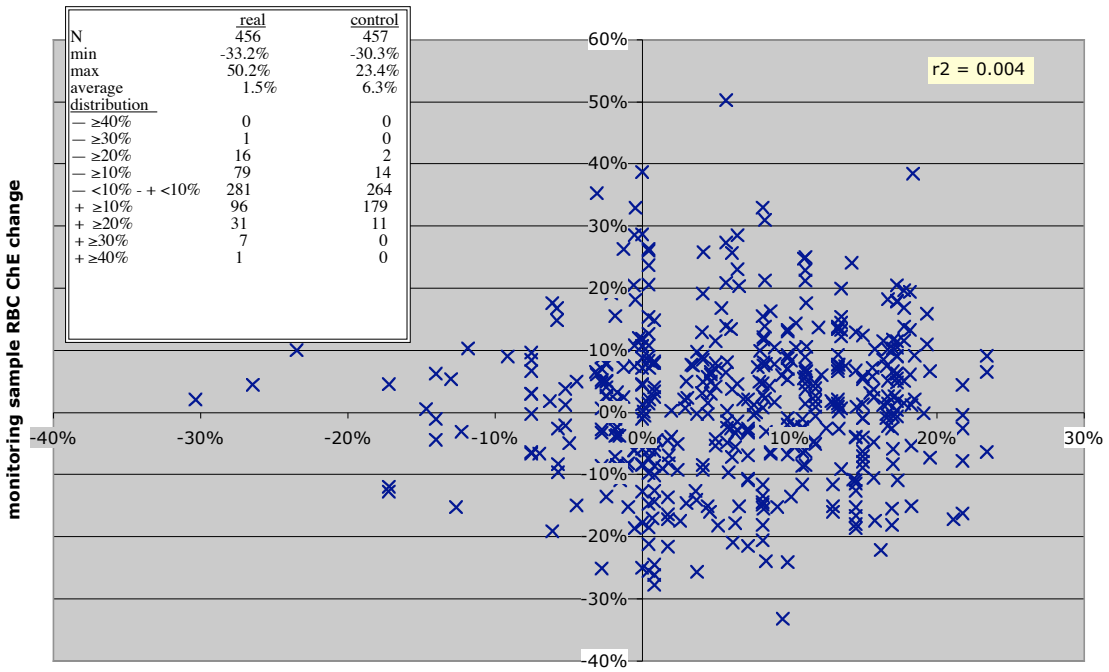


Figure 3.4: Relationship of RBC ChE changes between baseline and periodic tests for monitoring samples and HR RBC control samples

This analysis suggests that the finding of overall elevation in RBC ChE from baseline to follow-up in monitoring samples could be a laboratory artifact that either resulted from startup conditions or that could be corrected with method changes. It is clear that reducing between-batch or between-day variation in results and method sources of bias will be important to detect meaningful changes at the 20% depression level.

3.4 Summary

The Public Health lab successfully developed a routine assay to carry out the monitoring program objectives during year 1. Following the initial startup period, the lab produced results consistent with good laboratory practice, of acceptable precision, and of comparable quality to that of related monitoring programs. The lab also demonstrated the organizational ability to handle large sample loads under time pressure and to adjust to unanticipated needs to meet monitoring program requirements.

Despite QC results indicating acceptable overall precision and accuracy, there are indications of measurement bias for specific assay periods, which could lead to either over- or under-estimation of depression in RBC ChE data for individuals, but seems likely to lead to underestimation of exposures for the group as a whole. It is not clear whether this results from low baseline results, elevated follow-up sample results, or both. No final conclusions can be drawn on this point using 2004 data, but the need for further

improvement in precision and accuracy in the lab analysis is suggested. Given the possibility of a major confounder in the Year 1RBC results, statistical analysis of monitoring results as provided in Chapter 4 will emphasize serum data.

Beyond specific adjustments to current procedures identified in this section, the PH Lab is encouraged to reassess its overall methods in concert with experts in the field of enzymology and specifically cholinesterase enzyme characterization. The goals would be to optimize or customize the commercial package of reagents and instrumentation together with lab procedures for sample handling and preparation. The desired outcomes would be to reduce the sampling and analysis variability of ChE assays; to remove possible sources of bias; to develop more robust indicators of assay accuracy and stability for ongoing use; and to increase lab capacity if possible. The RBC assay in particular needs more refinement to reduce variability and bias to meet the needs of this monitoring program.

The need for more capacity is going to be increased by lowering the requirement of employers to designate possible monitoring enrollees based on 30 hours/month of handling versus 50 as is currently the case. Another factor that might increase the demand on the lab would be if a confirmatory test or repeat baseline were added, either for all subjects or conditioned on initial testing results.

CHAPTER 4: ANALYSIS OF CHOLINESTERASE MONITORING RESULTS

This section describes the results of the analysis of the cholinesterase monitoring data. The data were obtained from the Department of Labor and Industries after Labor and Industries supplemented a database originally assembled by the Department of Health. The database was provided with information on the results of the cholinesterase tests as well some demographic information and information on number of hours worked as reported by the employer and linked to the specific test (or subject). The data were provided to the committee from the Department of Labor and Industries in the form of an Excel spreadsheet.

4.1. Description of Monitoring Data from Year 1

Samples were sent into the laboratory over the season from three distinct population sources: 1) handlers who were being monitored for exposure under the ChE rule; 2) control subjects who were unexposed and whose samples were sent in by the Department of Labor and Industries disguised as handler samples; 3) donors recruited by the Washington Farm bureau whose blood samples were submitted as coming from pesticide handlers but which were in fact intended to serve a control group and quality control function. This group includes approximately 50 samples. Group 2 alone was relied upon for control group data, given uncertainties about the origin of group 3 samples.

Subsequent to the original analysis of data for this report, the scientific advisory team was informed that a fourth exposure population was included in group 1. Up to 76 samples were submitted by individuals who did not qualify for monitoring under the rule ("Other workers" in Table 4.0). Their exact exposure status is not precisely known, however analysis of their cholinesterase results indicates that 33 underwent a single follow-up test. None had more than a single follow-up test on record. Of these 33, none showed inhibition of either RBC ChE or serum ChE beyond 20%. They therefore contribute to the total number of tests done but do not contribute to the number of depressions. A complete summary of the updated data set is shown in Table 4.0 below. A total of 580 group 1 agricultural workers had at least one periodic test (either RBC ChE or serum ChE); 577 had both RBC and serum ChE test results. The total number of periodic tests (summed across all testing periods) for group 1, following exclusion of group 4, is 911.

Table 4.0. Number (percents) of tests for each population source - revised[9]

Subject Category	Number of Baselines	Number of 1st RBC ChE periodic tests	Number of 1st Serum ChE periodic tests
1. Handler	2611(RBC), 2591(serum)	579	578
2. Control (L&I)	58 (RBC & serum)	36	36
3. Control (other)	43 (RBC & serum)	16	16
4. Other workers	43 (RBC & serum)	33	33
Total	2755 (RBC), 2735 (serum)	664	663

The scientific advisory team chose to maintain the report results as they were initially presented and add to the report this explanation of the potential effect of removing these samples on the results rather than to reanalyze the entire data set. The continued inclusion of the results from these 33 workers will affect primarily the percentages calculated for depressions among workers who underwent follow-up testing. Were these workers excluded from calculations, the percentage of notifiable depressions (>20%) among eligible workers would rise from 12.89 % to 13.63% for serum ChE and from 3.6% to 3.8% for RBC ChE. Minor changes in other calculations would also occur but the effect would be small. With the exception of Table A2.8. and its accompanying discussion, the following description and analyses are based on the original group 1 data (which included group 4).

Three hundred seventy individual employers were identified as having had handlers participate in the monitoring program. A total of 2758 baseline samples were analyzed and the laboratory analyzed 664 initial periodic tests that included information on the population source. Red blood cell cholinesterase was analyzed for 612 pesticide handler periodic test values. Serum ChE was analyzed for 611 initial pesticide handler periodic test samples. Table 4.1 provides a breakdown of these baseline and initial periodic test values by category of subject population source.

Table 4.1. Number (percents) of tests for each population source- original

Subject Category	Number of Baselines	Number of 1st RBC ChE periodic test	Number of 1st Serum ChE periodic test
Handler	2655 (96)	612 (92.2)	611 (92.2)
Control (L&I)	54 (1.9)	36 (5.4)	36 (5.4)
Control (other)*	43 (1.5)	16 (2.4)	16 (2.4)
Total	2758	664	663

*Controls submitted by an independent group, not L&I.

[9] The entries in Table 4.0 were current as of February, 2005. Corrections to the overall database have been ongoping and result in some discrepancies between these number, the 11/04 draft version of this report, and the 1/05 L&I report cited in footnote 2.

The mean age of the handlers participating in the monitoring was 36 years of age. The population was 99% male and mostly Hispanic (Table 4.2).

Table 4.2. Ethnicity, and Gender of Handlers, Controls and Other

	Handler (%)		Control (%)		Other (%)		Unknown	
Gender	M	F	M	F	M	F	M	F
Hispanic	2459 (93)	0	48 (94)	0	9 (25)	0	4	0
Not Hispanic	167 (6.3)	5	3 (6)	3	26 (75)	6	2	0
Unknown	22 (<1)	0	0	0	0	2	0	0
Total	2652	5	52	3	35	8	6	0

Samples began to arrive in the laboratory in January of 2004. Samples continued to arrive through September of 2004. Table 4.3 shows the distribution of the months of arrival of the baseline and periodic test samples. The vast majority of baseline samples arrived in February and March with the majority of periodic sample arriving one month later. The huge number of samples arriving at once presented a significant challenge to the capabilities of the laboratory.

Table 4.3. Months of Submission of Samples

Month	Baselines (%)	1ST Periodic tests (%)
January	22 (0.8)	0
February	1039 (37.7)	0
March	1326 (48.1)	12 (0.4)
April	155 (5.6)	304 (11.0)
May	116 (4.2)	215 (7.8)
June	28 (1.0)	77 (2.8)
July	62 (2.2)	13 (0.5)
August	10 (0.4)	43 (1.6)
September	0	2 (0.1)
Total	2758	666*

* Two periodic test values were without population source designation

Table 4.4. Mean Baseline RBC ChE and Serum ChE Activity by Ethnicity in Handlers, Controls and Others

Ethnicity	Hispanic			Non Hispanic			Unknown		
Class	H	C	O	H	C	O	H	C	O
RBC ChE	12.23 (1.39)	12.64 (1.27)	12.32 (1.23)	12.42 (1.41)	11.39 (0.97)	12.93 (1.15)	11.99 (1.0)	NA	11.63 (0.30)
Serum ChE	4.71 (0.76)	4.04 (0.91)	4.26 (0.85)	4.48 (0.87)	4.2 (0.95)	4.44 (0.99)	4.39 (0.95)	NA	3.04 (0.86)

H = Handler, C = Control, O = Other, (S.D. in parentheses)

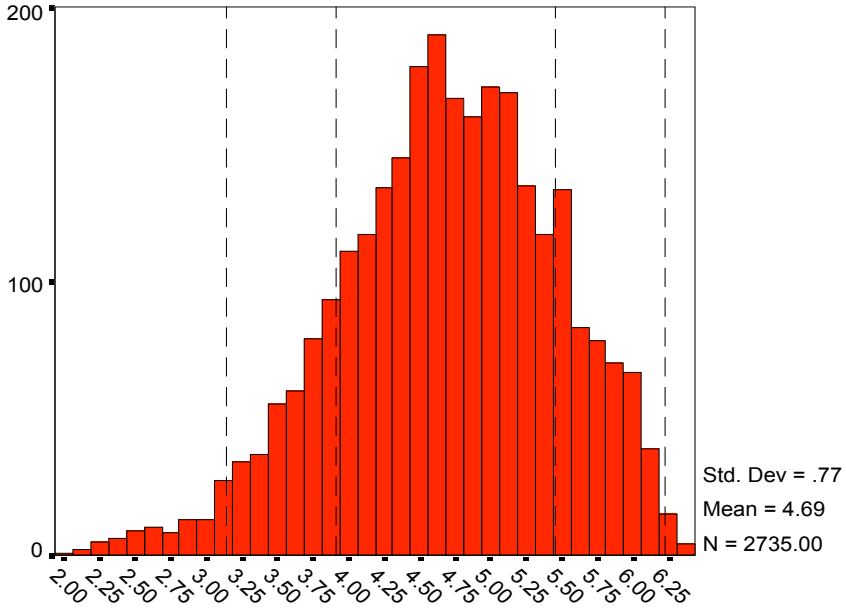
No significant difference was noted between handlers and controls on baseline RBC ChE. However, handlers had a significantly higher level of serum ChE activity on baseline testing compared to the 58 control samples from 29 individual subjects. Population variability was notably greater for Serum ChE (CV = 11-28%) than for RBC ChE (CV = 3 – 11%). Hispanics and non Hispanics had similar baseline RBC ChE levels but differed significantly in the serum ChE levels. Baseline serum ChE correlated with both age and ethnicity and remained true even when only handlers were included in the analysis. The significance of this finding is unclear. The tabulated data are presented in Table 4.4.

Table 4.5. Average value of baseline and 1st periodic test

Subject Category	Mean Baseline RBC ChE (n)	Mean Baseline Serum ChE (n)	Mean Periodic test 1 RBC ChE (n)	Mean Periodic test 1 Serum ChE (n)
Handler	12.23 (2652)	4.71 (2632)	12.42 (612)	4.32 (605)
Control L&I	12.51 (58)	4.11 (58)	12.37 (36)	4.10 (36)
Control other	12.74 (43)	4.33 (43)	12.37 (16)	4.07 (16)

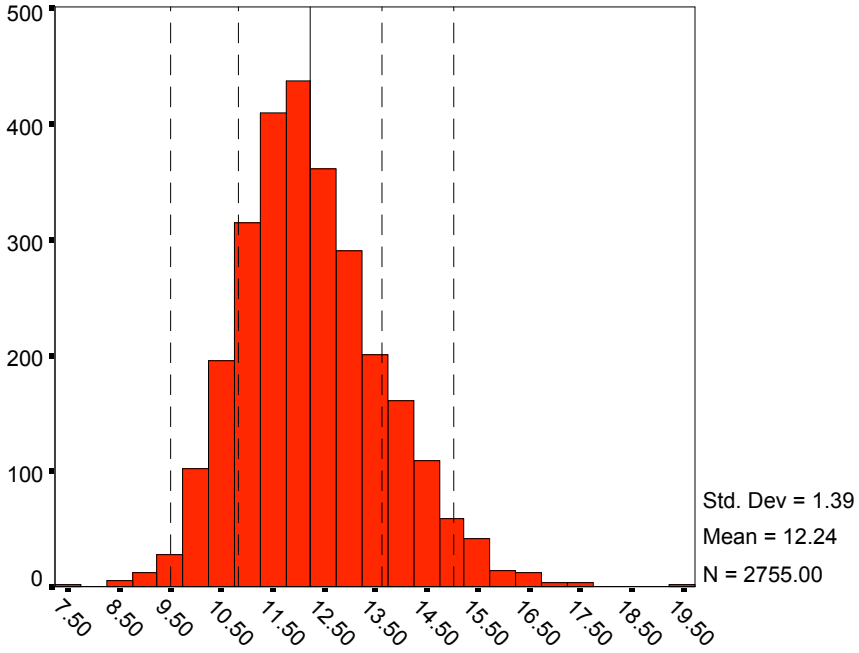
The data on baseline cholinesterase both serum ChE and RBC ChE are shown in Figures 4.1 and 4.2. A genetic variant of serum ChE activity exists in which baseline cholinesterase is consistently below the average population level. In the Anglo population this variant is predicted to be present in 3% of subjects. There are specific tests that explore whether an individual has a Serum ChE level that represents this variant. There is a possibility that some of the individuals among the lower end of the population represent these genetic variants. Ten subjects were identified with Serum ChE levels three or more standard deviations below the mean of the group. While these potentially might be due to causes such as chronic liver disease, use of cholinesterase inhibiting medications or even recent pesticide exposure, these extreme low values may also represent congenitally low serum ChE levels. All subjects with baseline Serum ChE that were 3 or more standard deviations below the mean for the group were among handlers.

Figure 4.1. Distribution of Serum ChE (Serum ChE) : All Participants.



Dotted lines on standard deviation units from mean.

Figure 4.2. Distribution of Acetylcholinesterase (RBC ChE) Baseline Values: All Participants



Dotted lines on standard deviation units from mean.

4.2. Changes in cholinesterase activity

Between March and September of 2004 periodic cholinesterase tests performed on handlers from across the state were submitted. Six hundred and twelve RBC ChE samples were compared to baselines and 605 Serum ChE samples were compared to baselines. There was an overall statistically significant drop in Serum ChE from baseline to periodic test when all 605 samples were averaged. The average change for the whole time period was a drop of 7.96% for Serum ChE. There was a statistically significant rise in RBC ChE across the same time period with the overall increase of 2.1%. Table 4.6 presents the distribution of changes across the months. Periodic test Serum ChE activities showed an average decrease beginning April and remaining negative until August, at which time only seven periodic test samples were submitted. Periodic test RBC ChE activity showed a rise beginning in March and continuing until August when again only 7 samples were submitted. It should be noted that beginning in May, average activity values for periodic test samples of RBC ChE differed little from baselines. The rise in average activity that occurred in April when 304 samples were submitted had the effect of moving the average over the season to a significant elevation. In the case of Serum ChE, each month from April through July, the period during which the bulk of samples were submitted, average Serum ChE values were depressed. This suggests an ongoing effect on the samples from some factors depressing the periodic test serum ChE but not RBC ChE values.

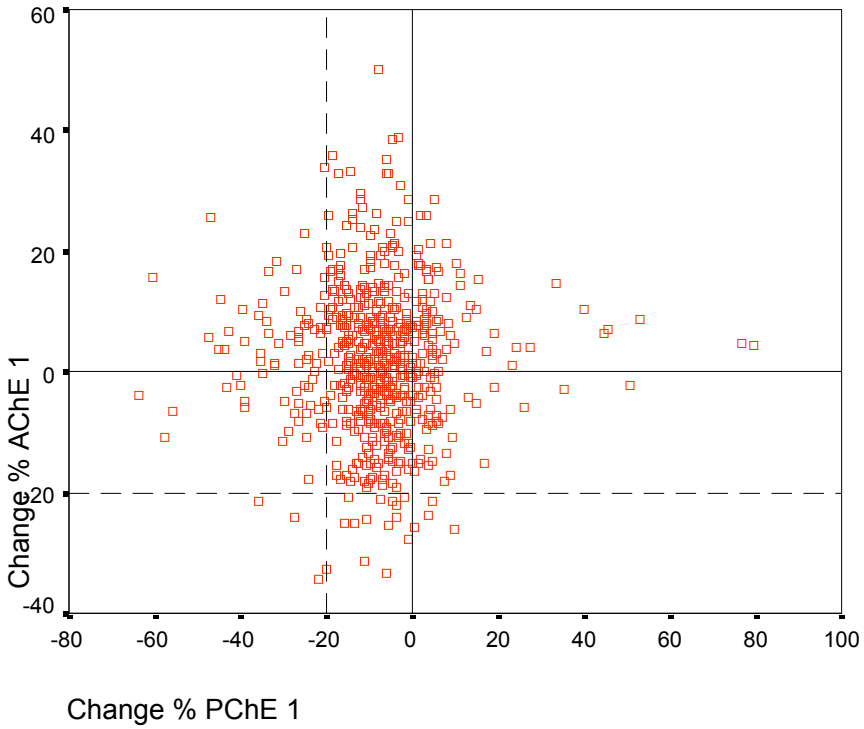
Table 4.6 . Month of Test by Mean Change from Baseline Among Handlers

Month of test	% Change from Baseline 1st RBC ChE Periodic test (n)	% Change in Baseline 1st Serum ChE Periodic test (n)
March	3.65 (12)	3.33 (12)
April	3.33 (304)	-9.6 (302)
May	0.93 (199)	-8.32 (195)
June	0.66 (76)	-4.18 (75)
July	1.56 (13)	-3.63 (13)
August	-2.62 (7)	4.54 (7)
September	-15.18 (1)	1.43 (1)
Mean Change	2.1 (612)	-7.96 (605)

A positive value indicates a rise in the mean

Figure 4.3 demonstrates the distribution of RBC ChE percent depression on first periodic test plotted against Serum ChE percent depression on first periodic test for handlers on first periodic test regardless of date. On the Serum ChE axis, the bulk of values are below the 0 (no difference) level, where as on the RBC ChE axis there is a tendency for the values to fall above the 0 reference line. This suggests and is borne out by other data such as that above that show that Serum ChE values on average were lower on periodic test while RBC ChE values on average were higher on periodic test.

Figure 4.3. Percent change during first periodic test among handlers in RBC ChE (x axis) versus serum ChE (y axis)



Dotted lines indicate 20% depression on both vertical and horizontal scales

The Table 4.7 presents the number of depressions (20% or greater depression from baseline) identified on each periodic test episode. The first column of both the Serum ChE and the RBC ChE halves of the table show the number of first time depressions with each of the repeat periodic tests. The second third and fourth etc. columns report on the 2nd, 3rd and 4th etc. times a single individual was found to have a depressed level. This provides some insight into the number of new depressed values vs. the number of repeat periodic tests showing another depressed value. There were a total of 100 first time serum ChE depressions and a total of 30 first time RBC ChE depressions out of a total of 612 handlers with tests. There were 54 subsequent depressed Serum ChE values but these were not new depressions. Additionally there were 19 subsequent RBC ChE depressed values among handlers with a previously identified depression. Available information for this report does not include details about whether or why these individuals had persistent depression. The possibility exists that some of this persistence of depression was due to artifactually elevated baselines or to non-pesticide causes for depression.

Table 4.7. Number and order of depressions (20% or more) among handlers by periodic test number

Periodic test number	cases of serum ChE depression >20%						cases of RBC ChE depression >20%					
	1st	2nd	3rd	4th	5th	6th	1st	2nd	3rd	4th	5th	6th
1st	76						22					
2nd	20	30					5	9				
3rd	4	3	13				2	4	6			
4th			2	3			1					
5th				1	1							
6th						1						
7th												
8th												

4.3. Hours of reported exposure.

A potentially important variable to be examined with respect to cholinesterase depression is the hours of work with pesticides covered by the monitoring rule. When the reports of hours are examined in detail there appear to be problems with the accuracy of these data. Hours of exposure for the 30 days prior to that test are reported for only about half of the pesticide handlers with periodic testing[10]. Analysis of the hours reported for whom we do have this information shows pattern which suggests that reporting employers may not have accurately reported actual hours of exposure. While reports are available for over 300 employees, these reports come from only fifty-eight employers. Of these 58, more than half reported at least two employees with the identical number of hour of exposure. One employer reported the same number of hours for seven employees. Four employers reported the same number of hours for 6 employees. Twenty seven of the 58 employers reported the same number of hours for two employees. The lack of independence of values makes use of these data as independent observations in analysis potentially misleading. For this reason these data were not used in analyses of Serum ChE or RBC ChE depression.

However, if the assumption is true that there is lack of independence in these data and that employers did some averaging when reporting values, some information may still be derived by a comparison of pesticide handlers when the data are grouped by the hours of exposure. Table 4.8 examines this approach, which shows the mean values for RBC ChE and serum ChE baseline and percent change with the first periodic test by groups of hours of reported exposure to covered pesticides. Using one way analysis of variance, there is no significant difference between values of any of these variables across reported groups of reported hours. However, when pesticide handlers with periodic test

[10] This was the case as of September/October of 2004 when these analyses were performed. An extended but still incomplete accounting of hours of exposure for periodic test samples has since been added to CMDS; some further analyses are included in the L&I report cited in footnote 2.

tests and no reported hours are also included in the analysis as a group, a very significant difference is present in the mean Serum ChE % change. Pesticide handlers with any reported hours as a group have a mean % Serum ChE change of -9.04 %. This was a greater degree of depression than pesticide handlers whose hours of work were not reported (-5.90%). This difference was very significant statistically ($p<0.01$). There was no significant difference in mean % RBC ChE change between the same groups. This does suggest that pesticide handlers who have reported hours may have had more exposure to covered pesticides than pesticide handlers without reported hours.

Table 4.8. Mean baseline and follow-up percent change, by hours of reported work

Hours of work	RBC ChE Baseline (n)	Serum ChE Baseline (n)	RBC ChE % Change (n)	* Serum ChE % Change (n)
None reported	12.30 (344)	4.60 (342)	0.93 (344)	-5.90 (341)
1-30	12.l5 (50)	4.60 (49)	1.65 (50)	-8.63 (49)
31-50	12.29 (104)	4.85 (104)	2.02 (104)	-10.63 (104)
51-100	12.25 (151)	4.75 (149)	3.08 (151	-8.69 (148)
100 +	12.29 (15)	4.56 (15)	4.81 (15)	-2.86 (15)
Total	12.30 (664)	4.68 (659)	1.73 (664)	-7.40 (657)

One-way Anova difference significant $p < 0.01$

4.4. Characterization of RBC ChE and Serum ChE Results for Alerted and Removed Pesticide handlers

A total of 612 pesticide handlers participated in at least one blood draw as a follow up to their baseline draw. Of this total, 612 had a validated test for RBC ChE, and 605 had a validated test for Serum ChE. Fewer pesticide handlers participated in subsequent follow up blood draws (Table 4.9).

The first follow up blood draw qualified 76 pesticide handlers (~13% of first periodic serum tests) for an "alert" because their serum ChE levels were depressed by greater than 20% from the initial baseline levels (Table 4.9). The first follow up measurements of RBC ChE revealed that 22 pesticide handlers (3.6% of first periodic RBC tests) should have received an alert because of depressions of more than 20% of baseline levels (Table 4.10). The proportion of pesticide handlers falling into an alert status owing to greater than 20% depression of RBC ChE or Serum ChE levels rose as fewer pesticide handlers received subsequent follow up blood draws (Table 4.9, 4.10, F2).

Twelve pesticide handlers qualified for work removal status on the basis of serum ChE levels in the first follow up blood sample (Table 4.9). Four pesticide handlers had first follow up RBC ChE levels sufficiently depressed (>30%) to place them on work removal status (Table 4.10).

Table 4.9 Number and Percentage of Periodic Serum ChE tests performed, number of tests with depression exceeding 20% and 40%

Periodic Test	# of Periodic Tests Performed	# of Tests >20% depression	# of Tests >40% depression
1	605	76	12
2	246	50	6
3	111	20	5
4	27	5	1
5	5	2	1
6	1	1	0
7	1	1	0
8	1	0	0

Table 4.10 Number and Percentage of Periodic RBC ChE tests performed, number of tests below 20% and 30% depressed

Periodic Test	# of Periodic Tests Performed	# of Tests >20% depression	# of Tests >30% depression
1	612	22	4
2	242	14	5
3	111	12	3
4	27	2	0
5	5	1	0
6	1	0	0
7	1	0	0
8	1	0	0

Over all eight periodic cholinesterase tests, a total of 130 pesticide handlers exhibited >20% depression of either RBC ChE or Serum ChE levels from baseline (Table 4.11). Of these, 78% resulted from Serum ChE test results and 22% from RBC ChE results. Of the 26 pesticide handlers (4.2% of total follow ups) exhibiting enzyme depressions warranting removal status, 62% had Serum ChE levels less than 60% of baseline, and 38% had RBC ChE levels less than 70% of baseline.

Nine of the 130 pesticide handlers had both RBC ChE and Serum ChE test results showing 20% or more depression from baseline levels. Of this group, five pesticide handlers qualified for work removal due solely to RBC ChE depressions greater than 30% from baseline.

Table 4.11. Overall number of pesticide handlers and proportion qualifying for alert status (>20% enzyme depression from baseline test levels) and work removal status (>30% depression in RBC ChE; >40% depression in Serum ChE).

Test	Number > 20% Depression From Baseline	No. Qualifying for Work Removal
RBC ChE	29	10
Serum ChE	101	16
Total RBC ChE and Serum ChE*	120	22
% of Total Pesticide handlers Participating (n=612)	19.6	3.6

*corrected for workers with both RBC and Serum ChE depressions

4.4.1 Work Place Characteristics

Most the of 612 pesticide handlers with at least one periodic blood test stayed with the same employer throughout the growing season. Sixty-one pesticide handlers were associated with a different employers for periodic test 1 and periodic test 2. Eight pesticide handlers had different employers for periodic test 1 and periodic test 2, but 6 of these 8 pesticide handlers had the same employer during periodic test 1 and periodic test 2. Five of the pesticide handlers with different employer ID's had cholinesterase levels depressed below 20% of baseline but none had depressions qualifying them for work removal.

A total of 51 alert and work removal interviews were conducted to ascertain pesticide use. Thus only ~40% of pesticide handlers on alert or removal status were covered in the survey of pesticide use.

Nearly all pesticide use was consistent with production of pome fruits (Table 4.12, 4.13). Four pesticides repeatedly showed up in the interview information: Sevin (carbaryl), Lorsban (chlorpyrifos), Carzol (formetanate), and Guthion (azinphos-methyl). One pesticide handler used diazinon and one pesticide handler had used Vydate (oxamyl).

The greatest proportion of the pesticide handlers qualifying for alert status had been using only one insecticide, Sevin or Lorsban (Table 4.12). Use of mixtures represented a significant but lower proportion of pesticides handled. The greatest proportion of pesticide handlers classified for work removal used a mixture of Sevin and an organophosphorous insecticide (Lorsban or Guthion) (Table 4.13).

Table 4.12 . Number of pesticide handlers exhibiting RBC ChE or Serum ChE levels requiring alert status and associated pesticide use (n=51 interviews)

Pesticide	RBC ChE Associated	Serum ChE Associated	Total Use	% of Interviews
Sevin/Sevin+Carzol *	5	17	22	43.1
Lorsban Alone	4	10	14	27.5
Sevin + OP	4	8	12	23.5
Lorsban + Other OP/Carzol	1	6	7	13.7
Guthion	1	0	1	2.0
Diazinon	0	1	1	2.0
Total Responses	15	42	57**	

*One of the pesticide handlers requiring an alert because of RBC ChE levels and three requiring an alert because of plasma levels used a combination of Sevin and Carzol (which are both methyl carbamate insecticides).

** Responses total more than 51 because some pesticide handlers' pesticide use was associated with both RBC ChE and Serum ChE levels that met the 20% depression from baseline criteria.

Table 4.13. Number of pesticide handlers exhibiting RBC ChE or Serum ChE levels requiring work removal and associated pesticide use (n=51 interviews)

Pesticide	RBC ChE Associated	Serum ChE Associated	Total	% of Interviews
Sevin/Sevin+Carzol *	2	3	5	9.8
Lorsban Alone	3	1	4	7.8
Sevin + OP	6	4	10	19.6
Lorsban + Carzol	0	1	1	2.0
Guthion	1	0	1	2.0
Total Responses	12	9	21	

* Two of the pesticide handlers requiring removal because of RBC ChE levels and one requiring removal because of plasma levels used a combination of Sevin and Carzol (which are both methyl carbamate insecticides).

4.5. Analysis of monitoring results with regard to assessing predictive power of ChE data

Statistical analyses were conducted on the baseline and first periodic test results for pesticide handlers to determine if there were significant differences between ChE activities at baseline versus periodic test, and to determine the amount of random scatter in results. This analysis of variance is detailed in Appendix 2. Key findings were:

- There was a highly significant drop in serum ChE activity levels from baseline to first periodic test among handlers. There was a highly significant variability in serum ChE levels among handlers as well. The within-person variability as a standard deviation after removing trend from baseline to first periodic test is

estimated to be 0.4495. This translates to a coefficient of variation at mean baseline level of approximately, 0.4495/4.7242=0.095 or 9.5%.

- There was a highly significant change in RBC ChE levels from baseline to first periodic test. This change represents an increase from baseline—not a decrease. There was also a highly significant variability in RBC ChE levels among subjects. The within-person variability as a standard deviation after removing trend from baseline to first periodic test is estimated to be 1.0805. This translates to a coefficient of variation at mean baseline level of approximately, 1.0805/12.2601=8.8%

Estimates of limiting values of false positive results were calculated using the number of occurrences of apparent depression form 2004 and the statistical analysis cited above. These estimates are fully presented in Appendix 2, but are summarized as follows:

- The likelihood of a false positive for unexposed workers is approximately 4-6% at the 20% depression recognition level, 0.3-0.6% at the 30% depression recognition level, and less than or equal to 0.02% at the 40% depression recognition level.
- The amount of true depression required to give a 75% chance of correctly recognizing ChE depression would be 27% ChE depression at the 20% depression recognition level, 36% ChE depression at the 30% depression recognition level, and 45-46% ChE depression at the 40% depression recognition level. If true exposures were less than these values, the likelihood of false negative results would exceed 25%.
- To achieve 95% likelihood of correctly classifying workers as having ChE depression, the required amount of true ChE depression would be 35-37% ChE depression at the 20% depression recognition level, 43-45% ChE depression at the 30% depression recognition level, and 51-53% ChE depression at the 40% depression recognition level. With true exposures this high, the likelihood of false negative results would be 5%.
- The number of cases of actual ChE depression that occurred in 2004 depends on the number of false negatives results that occurred. This is not known and can't be determined from monitoring data. However, assuming that the number was zero provides an estimate of the fewest cases of actual depression that is consistent with 2004 monitoring results. For serum ChE, this would be 55 cases of 91 apparent cases of depression at the >20% level, or about 6% of all periodic tests. Of the 12 cases of apparent serum ChE depression of >40%, all were true positives amounting to about 1.3% of all periodic tests. Given the likelihood that the number of false negative tests was greater than zero, rates of occurrence of ChE depressions would be expected to exceed these values.

4.6. Conclusions from data analysis:

There was a statistically significant decrease in serum ChE activity between baseline and (first) periodic test measurement in this group. This depression is not explainable by simple biological variability, laboratory error or laboratory methods drift. A major factor that resulted in this downward movement of periodic serum ChE values is likely to be pesticide exposure among monitored handlers.

For RBC ChE, there is a statistically significant increase in ChE activity from baseline to periodic test on a population-wide basis. No clearly identifiable biological or exposure phenomenon easily explains this finding. Further interpretation of RBC ChE as an indicator of pesticide exposures using Year 1 data is not justified, given this apparent confounder.

The difference in patterns of depression for Serum ChE versus RBC ChE may be a function of the pesticide to which pesticide handlers were predominantly exposed. Chlopyrifos preferentially depresses Serum ChE rather than RBC ChE and this is a commonly used pesticide in pome fruits in Washington State.

Within-person variability for this group in 2004 was about 10%CV for Serum ChE and slightly less for RBC ChE. This includes all sources of variation other than systematic changes between baseline and periodic test (which are presumed related to exposure): sampling, analysis, random biological variation, and random life-style factors.

Conclusions regarding the relationship between reported hours of pesticide handling and ChE depression were not possible, due to limitations in the information available for analysis at the time of this report .

Using estimates of within-person variability from 2004 Serum ChE data predicts that at most 31 of 155 positive tests at the 20% depression cutoff were incorrect, 2 of 64 positive tests at the 30% and 0.03 of 29 positive tests at the 40% cutoffs were incorrect. Therefore, a value that was 20% depressed from baseline had a greater than 80% probability of being depressed due to an actual change in the activity of the enzyme. For observations of depressions beyond 20%, the reliability increased significantly, at greater than 97% for 30% depression. For “exposure removal alert”-level depressions in particular, it appears that the serum ChE test is quite likely to correctly identify pesticide handlers with real depression. The actual rate of false positives could be significantly lower than this worst case but can’t be determined from available data.

The frequency of positive tests after correction for expected false positives can be determined to be at least 155/911 = 13.6%, 64/911 = 6.8%, or 29/911 = 3.2% for the entire group of periodic tests, if defined as 20%, 30% or 40% depression of serum ChE activity, respectively. This minimum value presumes no false negative results.

Reducing the number of false positive results by changing regulatory action limits will necessarily increase the expected number of false negative results. Another way of stating this is that the amount of true exposure and true ChE depression that it would take in order to have a high likelihood of detection would go up if the action limit used to classify exposure were raised. Moving the 20% threshold up to 30%, for example, would decrease the number of false positive cases from 6 per hundred to 0.6 per hundred, but would decrease chances of someone with true exposure being correctly classified. For workers with 40% true depression, changing the alert level from 20% depression to 30% depression would increase the missed cases from fewer than 3 per hundred to about 15 per hundred. The Monitoring Program as part of its regulatory decision making must weigh the cost of each of these outcomes, true positives vs. false positives and false negatives vs. true negatives.

CHAPTER 5: ASSESSMENT OF PROGRAM IMPLEMENTATION IN 2004

This section provides information related to the evaluation and implementation of the cholinesterase monitoring program. It focuses on four aspects of the cholinesterase monitoring program. They are:

- Assessing employer enrollment of pesticide handlers in the cholinesterase monitoring program;
- The timeliness of the cholinesterase monitoring system in processing samples and reporting results;
- WISHA consultation visits to employers as part of the cholinesterase monitoring program; and
- Survey results related to the medical providers knowledge, attitudes, beliefs and experiences regarding the cholinesterase monitoring program during 2004.

5.1. Employer Enrollment of Pesticide Handlers into the Cholinesterase Monitoring Program

In 2004, the cholinesterase monitoring rule required employers to enroll handlers in the cholinesterase medical monitoring program if the hours of organophosphate (OP) and N-methyl-carbamate exposure were expected to meet or exceed 50 hours during any consecutive thirty-day period. Handlers were referred to a health care provider for initial medical evaluation and consideration for inclusion in the cholinesterase testing program. The rule required baseline cholinesterase testing to be completed after at least a 30-day period during which the employee had not handled OP and N-methyl-carbamate pesticides.

The total number of handlers who participated in cholinesterase baseline testing was 2655. The number of employers referring handlers for testing was 370. The greatest number of handlers referred by one employer was 166. The median number of handlers referred was 3 and the mean was 7.2 workers per employer.

Table 5-1: Employers Referring ≥50 Pesticide Handlers for Baseline Cholinesterase Testing and Proportion with Periodic Testing

Grower Employer ID	Number of Workers with Baseline Tests	Number of Workers with Periodic Testing	**% Workers with Periodic Testing**
20	166	30	**18%**
24	121	33	**27%**
8	111	11	**10%**
247	93	12	**13%**
40	82	47	**57%**
21	71	0	**0%**
16	60	4	**7%**
193	55	31	**56%**

Eight employers referred 50 or more handlers for baseline testing (759 handlers; 28.6% of all workers referred for baseline testing). Of these 759 handlers, only 168 (22%) had at least one periodic cholinesterase monitoring test result following baseline testing.

Only 2 of the eight employers had more than 50% of the handlers participate in periodic testing after baseline test results were completed. Table 5-1 presents results for employers with 50 or more workers participating in baseline testing.

Forty-seven employers had 10 to 49 handlers participate in baseline testing (956 workers; 36% of all workers referred for baseline testing). Of those 956 workers referred only 279 (29.2%) had at least one periodic cholinesterase monitoring test result following baseline testing. Fourteen of the 47 employers had more than 50% of the workers submit to periodic testing after baseline test results were completed. Of the 47 employers, 20 had less than 10% of the workers with baseline testing requiring a periodic test result.

The remaining 315 employers accounted for 940 baseline tests and 159 periodic tests (17% periodic testing.)

Employer referrals for baseline cholinesterase testing appears to be significantly in excess of what might be reasonable given the requirements under the rule. L&I estimated in its Small Business Economic Impact Statement that 1100 handlers would be covered by the medical monitoring requirements of the rule in 2004 with a declination rate of approximately 15%. There are speculative reasons for the large number of baseline tests not subsequently followed with periodic testing. The employer may have misinterpreted requirements for baseline testing. Requirements involve testing for handlers above a threshold of exposure, with specified pesticide exposures (Toxicity Class I or II organophosphate and N-methyl-carbamate pesticides). While one factor could be the use of very conservative estimates of expected handling hours, it is recognized that many factors in the workplace and growing season can make accurate prediction of spraying activity difficult and uncertain. Regardless of the cause of the apparent over-testing at baseline, increased outreach to employers regarding those who should be referred for baseline testing seems appropriate before the next season commences. The unexpectedly large number of baseline tests also contributed to the laboratory's inability to analyze and report results of baseline tests in a timely manner, as discussed below.

5.2. The timeliness of the cholinesterase monitoring system in processing samples and reporting results

Timeliness of laboratory receiving and processing samples, and the reporting of the results to those who can prevent subsequent exposure and mitigate the potential of pesticide poisoning is an essential part of the cholinesterase monitoring system. Established time periods of handling and processing of lab specimens are specified in the Laboratory SOP. Timelines for reporting significant cholinesterase depressions were established by L&I. Table 5-2 provides the information for the time periods measured, the optimum timeliness of the reporting and the actual results for 2004 season.

Table 5.2: Time Periods for Selected Steps in Cholinesterase Monitoring System

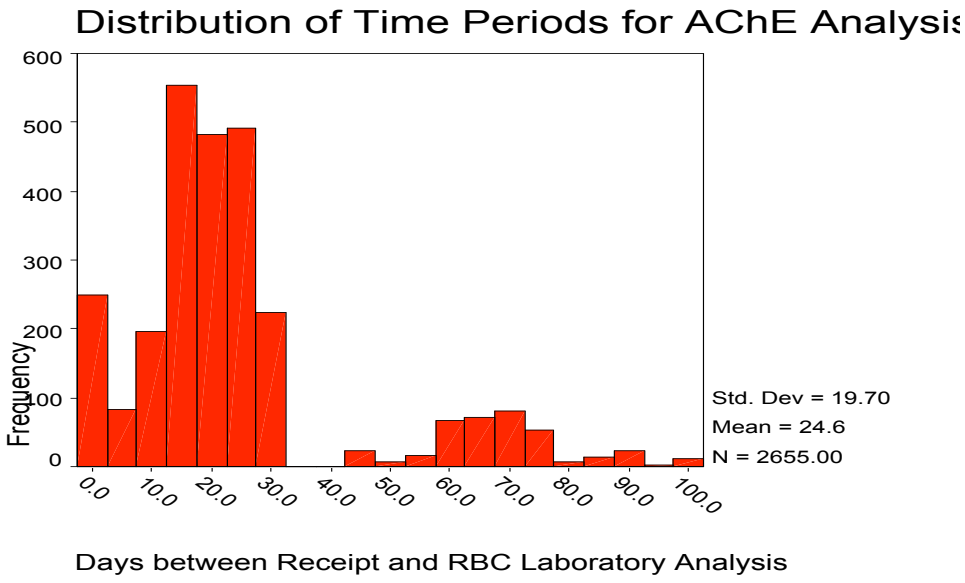
	Time Period	Expected Time (Days)	Actual Time
	Time Period for Baseline Testing		
A	Blood Draw and Receipt by PHL	1 – 1.5	1.1 day
B	PHL Receipt to Lab Testing	1†	24.6 days†
	Time Period for Periodic Tests		
C	Blood Draw and Receipt by PHL	1 – 1.5	1
D	PHL Receipt to Lab Testing	1†	1.1
E	Lab Testing to Mailing Result to Provider	2 from test date	3.6
	Time Period for Periodic Tests requiring Workplace Evaluation		
F	Lab Testing Data transfer to CMDS*	1	1
G	CMDS Notifying L&I P&TS**	1	1
H	L&I P&TS Inform Medical Provider**	3 from test date	3.6
I	L&I P&TS Inform WISHA Cons**	8 from test date	7.8
J	WISHA Cons. Notified to Workplace Consultation**	14	34.5
	Time Period for Periodic Tests Requiring Medical Removal		
K	CMDS Notifying L&I P&TS***	1	1
L	L&I P&TS Inform Medical Provider***	3 from test date	3.9
M	L&I P&TS Inform WISHA Cons***	8 from test date	7.2
N	WISHA Cons. Notified to Workplace Consultation***	7	35

* Periodic Testing only
** Periodic Testing with RBC ChE< 80% of Baseline and/or serum ChE< 80% of Baseline
*** Periodic Testing with RBC ChE< 70% Baseline and/or serum ChE< 60% Baseline
† The Lab SOP states that specimens must be analyzed within 48 hours of collection and if this is not possible must be frozen within that 48 hour time period in order to be tested at a later date (no longer than 4 weeks for RBC ChE and 6 months for plasma ChE). The SOP states that the lab should receive samples no later than 36 hours after collection. If this is changed to "Blood Draw to Lab Testing" then 2 days is OK. Otherwise the lab should routinely prepare the samples on the same day received.

Two problems associated with timeliness are apparent from Table 5.2. The laboratory analysis of baseline samples was delayed. Approximately 91% of all baseline laboratory samples were run beyond the two day goal (but only 11% of baselines exceeded the SOP holding time limit). The delay in analysis was the result of both a greater than expected number of baseline samples being received by the public health laboratory (PHL), and difficulties in operating the testing instrument due to lipids in the blood samples clogging a micropipette. These difficulties were resolved in March when the laboratory developed and tested a dilution procedure for the serum samples (See Chapter 3). The test result from a baseline sample has limited clinical utility and serves as a reference for subsequent testing. Overall the delays in analysis and reporting of the baseline result to the provider had little impact on the health care providers delivery of services to the worker. Based on the reduction in cholinesterase activity demonstrated in the retesting of a sample of baseline tests the possible impact of a delay in testing would be to under estimate the number of true depressions. The major impact that the delay in running baseline tests had was to erode the confidence of stakeholders in the competency of the

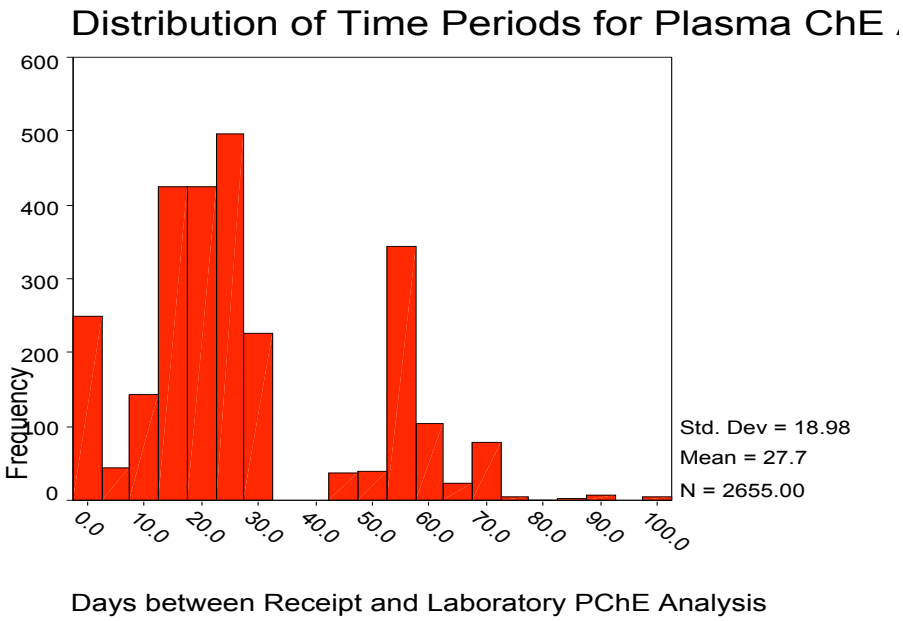
program as a whole. This erosion in confidence could have been lessened through better communication with providers, employers, and employees.

Figure 5.2.a



The distribution of the time periods between laboratory receipt of the baseline specimen and the subsequent analysis is bimodal, Figure 5.2a and 5.2b. Generally, laboratory

Figure 5.2.b



specimens received in January and February of 2004 reflect the group of specimens tested 40 to 100 days after receipt. Specimens received after the end of February were tested in a shorter time frame. This is due to the lab steadily eliminating the baseline

backlog and decreased numbers of baseline tests submitted over time. As shown in table 5.2 RBC ChE baseline tests were run on average 24.6 days after receipt. This is well within the maximum storage time range of 4 weeks specified in the Lab SOP. Storage time for frozen plasma cholinesterase samples was not an issue, as frozen samples remain stable for up to 6 months.

5.3. WISHA consultation visits to employers as part of the cholinesterase monitoring program

Based on consultation data for nineteen site visits conducted at the time of this report preparation.

Field data on pesticide handlers who had experienced a cholinesterase depression >20% from their baseline was gathered through the WISHA consultation program. The surveillance protocol and data gathering tools are contained in WISHA Regional Directive (WRD) 33.27 Cholinesterase Depression. All regional industrial hygiene consultants involved in data gathering (primarily regions 3 and 5) received training on the Cholinesterase Monitoring rule prior to the start of pesticide application season. Specific data related to the Cholinesterase Monitoring rule and pesticide exposure was gathered during the conduct of a routine pesticide worker protection program consultation activity. Pesticide handlers with cholinesterase depression >20% from baseline were interviewed whenever possible.

A total of 39 employers had at least one pesticide handler with a cholinesterase depression >20% from baseline. Fourteen of these had at least one employee with a cholinesterase depression requiring temporary removal from handling duties and other potential exposures to cholinesterase-inhibiting pesticides. All were contained in Region 5, which is composed of West Adams, Benton, Chelan, Columbia, Douglas, Franklin, Grant, Kittitas, Okanogan, Walla Walla, and Yakima counties.

L&I established a goal of having the region make initial contact with the employer within 3 days of receiving a consultation referral from WISHA Policy & Technical Services (8 days after laboratory testing). Consultation visits were then scheduled as soon as possible, at the employer's convenience. Consultation visits that involved employees who had cholinesterase depression to the exposure removal level were prioritized. The average length of time from referral to consultation visit was 34.5 days.

The time between identification of a significant ChE depression and conduction of a WISHA consultation evaluation was lengthy. The reasons for this delay results from 1) L&I delaying initial employer contact by several days in order to allow medical provider notification and the employer to have an interval period to address potential workplace pesticide exposures, 2) inadequate contact information provided by the employer, 3) L&I contacting the employer prior to the employer receiving a recommendation from the medical provider, and 4) difficulties scheduling consultation visits in a timely manner.

The means used to obtain employer contact and location information was to gather this information on the laboratory request form. In the majority of cases this provided an appropriate employer contact. However, in a few cases the wrong telephone number, employer contact, or growing site was provided. This resulted in delays in scheduling consultation visits.

Due to concerns about medical confidentiality the regional consultants were not given the names and specific test data on individual employees. This information was obtained through review of the documentation required in the Cholinesterase Monitoring Rule, Chapter 296-307-14835, and employer and employee interviews. The WISHA Occupational Nurse Consultant was available to clarify any discrepancies in the referral information and documentation maintained by the employer. There were several occasions, early on in the program, where WISHA consultation contacted the employer about a cholinesterase depression before the employer had received a recommendation from the medical provider. This situation caused some confusion and was corrected by delaying the referral for several days after receiving the notification of a significant cholinesterase depression from the Department of Health Cholinesterase Monitoring Data System (CMDS). This delay allowed time for the medical provider to review the test result and provide a recommendation to the employer before the initial consultation contact.

Common situations encountered that required intervention from the Occupational Nurse consultant included:

- L&I contact with the employer prior to the employer receiving notification from the medical provider.
- Clarifying the number of employees covered by the referral.
- Verifying test results and cholinesterase depression levels
- Clarifying cholinesterase depression action levels and recommendations for medical follow-up.
- Acting as a conduit between the employer and medical provider in regards to receipt of test results and scheduling follow-up monitoring for employees with cholinesterase depression to the exposure removal level.

All 39 employers granted consultation visits. As of this writing consultation summaries are available for 21 growing sites (19 employers) and include work practice evaluations for 37 individual employees. Spanish interpreters were utilized when necessary to interview employees.

All pesticide handlers evaluated applied covered pesticides and had experienced a cholinesterase depression >20% from their baseline. Most handlers also mixed and loaded cholinesterase-inhibiting pesticides, and to some degree cleaned/maintained application equipment and personal protective equipment. The vast majority of these pesticide handlers work in the tree fruit industry. Only two employees were involved in applying pesticides to field crops (”potatoes” and “row crops”). Another two applied pesticides to grapes, although they also applied pesticides to fruit tree crops.

There have been no accepted industrial insurance claims related to the cholinesterase monitoring program. Only one employee reported experiencing symptoms associated with cholinergic poisoning. This employee reported experiencing transient dizziness and nausea when applying covered pesticides but never reported this to his employer. This employee also reported not performing fit checks when donning his respirator and feeling a mist when applying pesticides.

Table 5.3 Covered pesticides reported

N-methyl-carbamates	Organophosphates
Carbaryl	Azinphosmethyl
Carzol	Chlorpyrifos
Oxamyl	Phosmet
	Dimethoate
	Diazinon

Airblast spraying is reported as the most common method of pesticide application. This is to be expected, as it is the most common method of pesticide application used in the tree fruit industry in this area. Most applicators use a half-face mask type respirator. The use of a half-face mask respirator may result in increased facial exposure. However, many of these employers require that pesticide handlers wear a respirator even when applying pesticides such as chlorpyrifos, which does not require respirator use at all. Deficiencies in equipment cleaning/maintenance and personal hygiene (mixing/loading/ applying and personal protection) activities were also identified during the consultation visits. No specific acute exposure episodes were identified.

Most employers maintained appropriate pesticide worker protection programs. However, there were some hazards cited during the consultation visits, these included:

- Lack of an adequate respiratory protection program
- No change out schedule for respirator cartridges
- Inappropriate respirator storage
- Lack of an adequate eyewash station at mixing and loading locations
- Failure to post the EPA registration number and active pesticide ingredient when required.
- Improper storage of pesticide containers.
- Failure to decontaminate application equipment and personal protective equipment after applying pesticides.

5.4. Survey results related to the medical providers knowledge, attitudes, beliefs and experiences regarding the cholinesterase monitoring program during 2004

5.4.1. Background

At the outset of the cholinesterase monitoring program 51 facilities indicated they might offer ChE monitoring services, but 7 proved unable to prepare in time to offer services during 2004. As a result 44 facilities received health care provider surveys, and

17 (39%) returned surveys for analysis. These 17 responding clinics accounted for approximately 85% of tests conducted. Nine of the 18 clinics had at least one depressed cholinesterase level, and these 9 clinics had 295 total tests showing depressions >20% (76 % of the total). Four additional clinics returned forms indicating that they had not received any requests for cholinesterase testing and did not respond to the survey questions. Staff completing the surveys included 10 registered nurses, 4 clinic managers, 5 physicians, and 1 lab manager (Three clinics had more than one respondent)

The facilities included 8 occupational health clinics, 2 hospitals, 5 community health or rural health clinics, and 2 private medical practices [**Q1**]. The facilities reported that employers became aware of their participation from multiple sources; these included advertising (41%), word of mouth (65%), existing relationships with an occupational health provider (59%), referral by other health care providers (18%), referral by farm organizations (18), from the Labor & Industries (L&I) web site (18%), or other sources (12%) [**Q2**]. The majority of the facilities (66.6%) reported that they initially were informed of the cholinesterase monitoring rule after being contacted directly by the Department of Labor and Industries [**Q3**].

Nearly all of [16 (94%)] of the 18 facilities responding to a survey query regarding the level of employer requests to provide medical monitoring, reported that they did not receive more requests than it proved possible to handle [**Q5**]. A high percentage [15 (88%)] also agreed that the demand for medical monitors did not exceeded the number available in their local areas [**Q6**].

5.4.2. Effectiveness of cholinesterase monitoring program

Sixteen facilities responded regarding the effectiveness of the cholinesterase monitoring program [**Q7**]. Six responded that the monitoring was effective, citing, for example, that patients with depressed ChE levels were identified. Three did not think the program was effective citing for, example, concerns that relatively few workers returned for periodic testing. Eight stated that they didn't know or that it was too early to tell. Some additional comments included:

> "Employees continue to fear loss of job"
>
> "Employers are getting around the ruling by decreasing the hours of exposure"
>
> "With a 6 month delay in getting results lots of people never bothered with retesting?"
>
> "Less than 20% of employees who had a baseline done with came back for further testing. Of the employees we tested, 40% had a decrease in [from?] their baseline"
>
> "The intent of the program is good. I worry about the participation because the growers who are doing their best to comply are the ones that handle the pesticides

cautiously to start with. The "rogue" groups likely will not participate until they are fined."

A related question [**Q21**] regarding the benefits of the program drew 39 responses from the 17 responding facilities, shown in the table below:

Possible benefit	**# of responses**	**% of total**
None	1	2.5
Prevention of pesticide illnesses	7	18
Increased awareness of chemicals in the workplace	9	23
Improved workplace safety	10	26
Increased community awareness	4	10
Other	8	20.5

5.4.3. Obstacles to providing monitoring services

All 18 responding facilities commented on obstacles to providing monitoring services they encountered [**Q8**] Only five facilities (29%) of the 17 responding facilities reported perceived problems with employer compliance, citing concerns such as employers not understanding the program or not being committed to the program.

Patient compliance was mentioned as an obstacle by four (23.5%) of the 17 facilities. Specific comments included:

> "We have patients who refused testing despite the fact that they received adequate instructions about the significance of testing. We realized that in some cases patients were afraid of abnormal results that would cause them to lose their jobs"

> "Patients not accountable for ensuring collection"

Seven facilities (41%) reported problems with laboratory services. The most commonly expressed concern was the time delay in reporting baseline results back to the provider. (See section 5.2.) Several facilities also expressed difficulty, inconvenience and cost associated with shipping samples to the laboratory.

Three facilities (18%) complained of difficulties in understanding rule requirements and a single facility complained of difficulty in communicating with L&I.

5.4.4. Informed consent and patient participation

Eleven (65%) of the participating facilities reported no difficulties with informed consent [**Q9**], two (12%) reported difficulty with language or communication, four (22%) reported that some patients had fear of needles or fear of having blood drawn. Two (11%) reported that difficulties with informed consent arose because of employer involvement.

Fifteen facilities reported on patients who declined participation in the testing program [**Q10**]. Ten (59%) of these 15 reported that some patients declined participation. Employees declined due to perceived employer pressure, fear of needles or having a blood sample drawn, and fear of retribution if the level was abnormal. One respondent felt the program was 'invasive', another felt the program was 'stupid' and two respondents felt that there was no need since 'working for years without an illness.'

5.4.5. Follow-up & notification

Nine (53%) of the facilities reported that they had followed up with employers to determine if their recommendations were being followed [**Q11**]. Specific comments indicated this was done by either verbal or written contact. No comments were made regarding perceived lack of compliance with medical recommendations.

Fifteen facilities reported whom they notified of test results: eight (47%) indicated that they notified the employer and seven (41%) indicated that they notified both the employer and the employee [**Q12**].

Fifteen facilities responded to a query regarding the average time delay between receiving ChE results from L&I and notification of employers or employees [**Q13**]: four (23.5%) indicated it took 1 or 1-2 days; seven (41%) indicated it took 2 days; three (18%) indicated it required 3 days; and one indicated that it took on average 4 days. Specific comments on this point included that the results were "reported same day as received" and "We still do not have all baseline results".

5.4.6. Evaluation of abnormal results

Thirteen facilities responded to a query regarding evaluation of patients for possible non-occupational causes for cholinesterase depression [**Q14**]. Three (18%) responded that they had, providing commentary indicated below:

> "We followed a patient with ChE depression. Patient had recent pneumonia that also contributed to the depression"
>
> "Not through WA program"
>
> "Medications from Mexico"
>
> "Recreational drug use suspected, no proof"
>
> "Before testing I evaluated all employees for medical conditions, medications, etc."

5.4.7. ***Follow-up evaluations for cholinesterase depression requiring medical removal***

In response to an open-ended question about procedures for follow-up of cholinesterase depression requiring removal from the work place [**Q15**] almost all providers indicated some form of retesting, either periodic or not, as a means for follow-up.

Fifteen facilities responded regarding employees who did not return for scheduled testing [**Q16**]. Eight (47%) indicated that they contacted the employer, five (29%) indicated that they would contact both the employer and employee and two (12%) indicated that they did not respond at all.

5.4.8. Symptomatic illness

Seventeen facilities responded regarding patients who showed symptoms of pesticide poisoning [**Q17**]. One responded positively, citing worker symptoms including nausea, headache, and dizziness.

5.4.9. Training for providers regarding cholinesterase monitoring

A majority (88%) of the facilities participating in the survey reported that they had the "Guidelines for Health Care Providers" manual prepared by L&I and the Pacific Northwest Agricultural Safety & Health Center (PNASH) [**Q4**]. Specific individual comments on the manual were generally positive, describing it as "clear" or helpful. Negative comments included descriptions of the manual as too complicated and bulky, with "too many items left to interpretation" or indicated a need to make the manual more concise.

Seventeen facilities responded regarding participation in training on the role and responsibilities of medical monitors [**Q18**]. Sixteen clinics responded regarding the best method for providers to obtain training [**Q19**]. Seven responses (41%) indicated that the "Guidelines for Health Care Providers" manual was sufficient; four (23.5%) indicated a desire for a self-study training course, seven (41%) expressed a desire for training presentations at their facilities, six (35%) indicated a desire for County/state-wide training courses.

Participating facilities gave 94 responses regarding specific areas to be covered in the training [**Q20**], shown in the table below:

Desired training topic	**#**	**% of total**
Rule requirements	13	14
Laboratory procedures	11	13
Establishing baselines	12	14
Periodic testing	13	13
Computing monitoring results as a percentage of baseline	9	10
Making recommendations based on test results	16	17

Follow-up testing of employees with significant cholinesterase depression	11	11
Evaluation of pesticide illness	9	9
Total	94	100

5.4.10. Change in attitudes towards the program

Fifteen facilities responded to a query [**Q22**] regarding changes in attitude towards ChE monitoring from the beginning to the end of the program; five (29%) indicated their attitudes had changed and eleven (65%) indicated they had no change in attitude. Specific responses included:

> "ChE monitoring is only one parameter to measure toxicity to pesticides. Long-term effects are not evaluated and there is no test to detect them."
>
> “I think that it is a flop”
>
> “Lots of paperwork and transport hassles living in a rural area”
>
> "Reaffirmed that there was not a problem, pesticide handling has been appropriately handled all along by the majority of employers and employees"
>
> “Understand the importance of testing”
>
> “Not much”

Additional comments [**Q21**]

> "How about creating an online calculator for ChE level change computation?”
>
> “Much lower turnout than expected for this area”

CHAPTER 6: RECOMMENDATIONS AND ISSUES

6.1. Overall program design

L&I / employers: In order to reduce the enrollment and baseline testing of workers who do not qualify for subsequent periodic testing, increased outreach before the next season commences to assist growers to identify only those workers most likely to meet the requirements for testing is needed. The outreach activity should occur before the start of the next season.

L&I / WDOH: The system of reporting results to medical providers needs reconsideration. As a special practice during the initial monitoring year, L&I notified providers whenever an alert level of ChE depression was encountered. Otherwise, results were communicated by US mail after the samples were completed. During the backlog phase, baseline samples were given lower priority until a related periodic test sample was received. As a consequence, providers had some samples submitted for a month or more with no response while results from other submissions were reported by telephone in only a few days. This seemed to create a lack of confidence in the lab's effectiveness. A second problem was that the Rule called for providers to be the parties responsible for relating serial tests and recognizing when pesticide handlers may have had ChE depressions above the alerting thresholds. However, this function was pre-empted by L&I computing depressions and notifying providers. More thought needs to be given to the competing needs for rapid recognition of depressions versus the need to engage providers in case ascertainment and follow-up, while assuring timely flow of information among employer, worker, health care provider, laboratory staff, monitoring program staff, and L&I personnel such as field consultants. The Committee recommends that L&I evaluate the effect of changes in reporting schema, if any are implemented.

L&I / WDOH: Continued use of a single testing methodology, and use of a single laboratory for all individual employee tests, is desirable in order to minimize extraneous differences within laboratories.

6.2. Program development

L&I: Stress a collaborative relationship between the provider, employer and employee in assuring that testing schedules are adhered to, test results and recommendations are conveyed in a timely manner, provider recommendations are followed, medical examinations are provided when indicated, etc.

L&I/WDOH: Evaluate laboratory resources necessary to process samples in a timely manner.

L&I: An evaluation plan for each aspect of the program (including the roles and responsibilities of employers, providers, handlers, and program personnel) should be developed. This will require consideration of what measures of effectiveness can be identified and for which data can be collected.

Issue: A method to assess the quality of data regarding hours of pesticide use is lacking. Some patterns in reported hours were noted that suggest that some estimates of worker handling hours might be imprecise or mis-specified.

6.3. Enrollment

L&I: Continue outreach to health care providers, employers and workers regarding the program to facilitate dissemination of information under the cholinesterase rule.
Growers: Try to identify more precisely handlers that are required to participate in the program so that a larger proportion of handlers receiving baseline tests also receive periodic tests.

6.4. Sample collection

L&I / WDOH: Continued educational outreach to providers to emphasize the importance of adhering to protocols for sample collection, labeling, and shipment in encouraged.

L&I / WDOH: Devise improved sample submission procedures that reduce or eliminate the need for hand matching of samples to handlers. A code identifier system with a unique ID to be used for each handler should be developed and applied. Providing pre-made labels for serial samples from a single handler upon enrollment in the monitoring program is a possible approach.

L&I: Encourage providers to repeat testing as soon as possible whenever a questionable test result is received.

6.5. Laboratory analysis

L&I: The blinded field QC sample submission activity should continue on an ongoing basis. L&I staff are encouraged to review QC results as they are reported and to respond if QC indicators suggest performance problems.

WDOH / L&I: The need for expansion of laboratory capacity and additional low temperature sample storage capacity in preparation for the 2005 growing season should be considered.

WDOH: When receiving shipments where the integrity of samples or the successful adherence to collection and shipping protocols appears questionable, sample rejection is recommended.

WDOH: Consider adding hemoglobin determination on each sample, to directly correct for RBC content in the sample and for volumetric error during pipetting of packed red blood cells in the RBC assay.

WDOH: Reconsider the use of a single enzyme substrate, acetylthiocholine, to assess both RBC ChE activity and serum cholinesterase activity. This substrate, although more

convenient in that it allows both assays to use the same test reagents, is not the optimum choice for the serum assay.

WDOH: Develop a quality control checklist for validating data, even if this is simply a formalization of review procedures already in place. This checklist could be used to document the review and acceptance of each set of measurements.

WDOH: Avoid hand transcription of lab results if electronic transfer is feasible.
Issue: Other issues that might result in changes in SOP include the exclusion of samples with evident hemolysis (disruption of red cells) affecting serum, and the use of hemolyzed but not solubilized RBC samples for analysis. Since the RBC enzyme is bound to cell membranes, dissolving those membranes using surfactant results in better sample homogeneity and greater assay precision. None of these issues clearly indicate major or known flaws in the data collected according to data analyses performed thus far, but do represent possible ways of reducing variability or bias.

WDOH: Beyond specific adjustments to current procedures identified in this section, the Public Health Laboratory is encouraged to reassess its overall methods in concert with experts in the field of enzymology and specifically cholinesterase enzyme characterization. The goals would be to optimize or customize the commercial package of reagents and instrumentation together with lab procedures for sample handling and preparation. The desired outcomes would be to reduce the sampling and analysis variability of ChE assays; to remove possible sources of bias; to develop more robust indicators of assay accuracy and stability for ongoing use; and to increase lab capacity if possible.

Issue: There was unexplained apparent bias in 2004 RBC data, that would lead to under-recognition of depression in RBC. This finding was true even for samples that were not affected by extended holding times. The apparent increase in RBC ChE activity from baseline to periodic tests seen for handlers as a group may suggest that RBC baselines were themselves depressed, but this would differ from the indications based on serum data. No clearly identifiable biological or exposure phenomenon easily explains this finding. However, depression of serum ChE with little or no RBC depression may be a function of the pesticide to which pesticide handlers were predominantly exposed. Chlopyrifos preferentially depresses serum ChE rather than RBC ChE and this is a commonly used pesticide in pome fruits in Washington State.

Issue: There is an ongoing need for a reference material for the RBC ChE assay; among existing reference materials, some show poor behavior compared to others.

Issue: Routine interlaboratory comparisons are needed for ChE assays. These would need to be devised to compare baseline-periodic sample activity differences rather than absolute cholinesterase activities.

Issue: In order to reduce uncertainty and false positives or in some cases false negative results, additional laboratory measures such as confirmatory testing or second baseline

samples might be useful. The procedures to be used and the feasibility and benefit of adding such measures would need to be evaluated.

6.6. Data analysis and interpretation

WDOH / L&I: Modification of CMDS to support data flags for questionable results based on field or laboratory indicators should be considered.

L&I / Growers: Improved collection of information describing hours of pesticide handling for each periodic test is needed in order to better examine the relationship between workplace conditions and risk of exposure.

Issue: To rigorously test the association between hours of handling and risk of ChE depression, a range of handling hours is needed. Establishment of a control group from the same general workplaces, but who do not perform direct handling of pesticides might be considered.

Issue: Simple compilation of hours of pesticide handling may not be a strong predictor of risk of exposure; additional information regarding identify of the pesticide and formulation and/or the nature of pesticide handling activities may be needed in the future.

Issue: Rising RBC ChE levels for some time periods are unexplained.

Issue: The rate of false negative results can not be determined from monitoring data.

Issue: Individuals with atypical ChE levels due to genetic factors, health status, lifestyle factors or extraneous exposures to ChE-inhibiting substances – how to identify and follow up?

Issue: Individuals with persistent (non-recovering) ChE depression. Some handlers with removal-level RBC depressions showed continuing low ChE compared with baseline. It is possible that some of the persistent depressions were due to erroneously elevated baselines or to depression from causes other than workplace pesticide exposures.

Issue: While the rate of false positive results (for the population tested) is at or below 6%, the fraction of apparent positive results that are false positives at the 20% depression level could be as high as 1 in 3. The alternatives to this situation are: (1) reduce overall imprecision of ChE data through improvements in sample collection and analysis methods; or (2) raise action thresholds above 20%, with the concurrent effect of increasing the frequency of false negative results.

6.7. Exposure response

L&I: Additional training to health care providers regarding the purpose of the program and the medical evaluation of workers with a depressed cholinesterase.

L&I: Encourage providers to access support resources regarding the cholinesterase medical monitoring program.

Issue: Timeliness of notifications as noted under "Overall Program Design" is a key element of exposure response.

L&I: Improve timeliness of WISHA consultation activities.

L&I: If resources allow, increase the number of WISHA consultation inspections to identify 1 common exposure scenarios and determination of health effects of pesticide exposure.

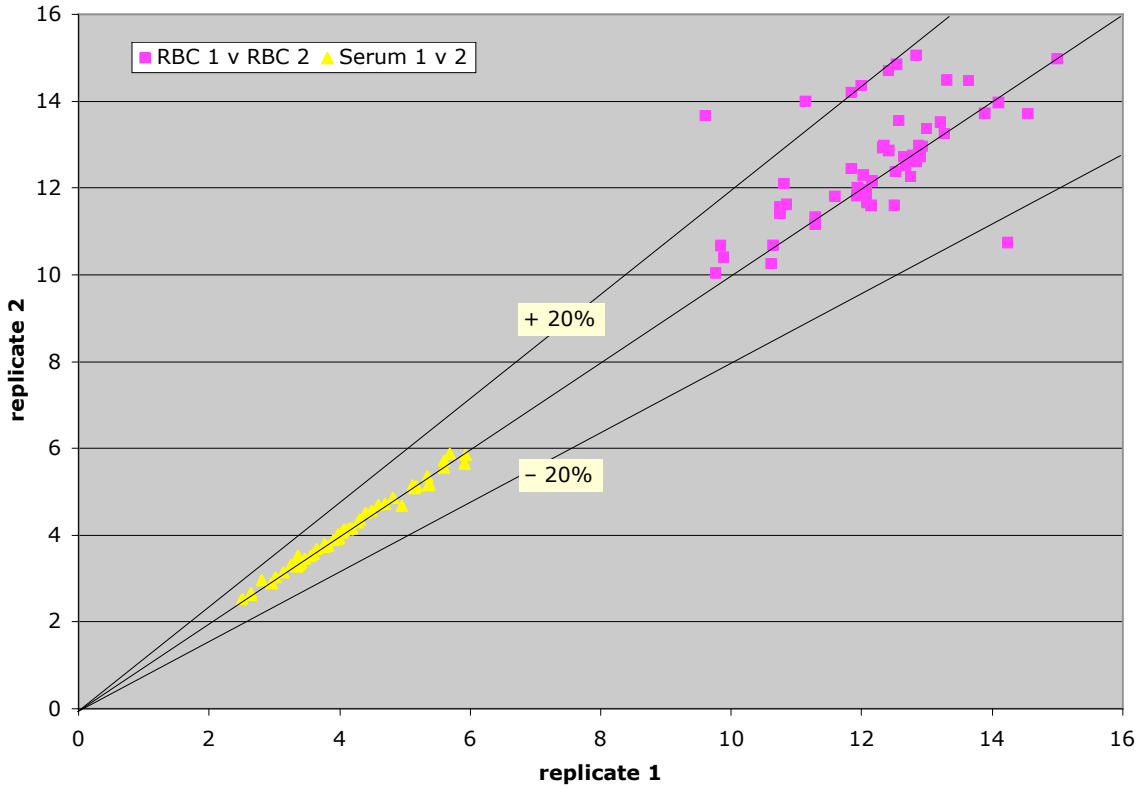
L&I: Consultation services should be performed systematically to ensure data collection is uniform. If possible, develop more quantitative procedures to be included in the standardized checklist for exposure assessment used for WISHA consultation inspections.

Appendix 1: Field replicate QC Data

Replicate precision for blind duplicates

Samples collected from unexposed volunteers were submitted as duplicates under alias subject names. Thirty pairs were collected initially and 23 pairs from the same pool of donors were collected at a subsequent sampling. Comparison of agreement for replicate pairs is shown in Figure A1.1 and in Table A1.1. No comparison between initial sampling and re-sampling by individual is made for these data.

Figure A1.1. Field QC data (blind replicate samples)



The raw data and summary statistics for these data set are shown in Table A1.2. The measure of agreement used, relative percent difference, differs slightly from that used by L&I to describe these results in its report to the legislature (January, 2005).

Appendix 1: Field Replicate QC Data

Table A2.1. QC Summary, blind duplicate samples

provi	Sample ID	Date	RBC 1a	RBC 1b	*RPD	Plasma 1a	Plasma 1b	*RPD
1	1	7/17/04	13.64	14.48	6.0%	3.65	3.68	0.8%
1	1	8/24/04	12.9	12.73	1.3%	3.31	3.3	0.3%
1	2	7/17/04	12.93	12.96	0.2%	2.64	2.62	0.8%
1	2	8/24/04	12.58	13.55	7.4%	2.8	2.95	5.2%
1	3	7/17/04	12.41	14.7	16.9%	3.9	3.87	0.8%
1	3	8/24/04	11.59	11.81	1.9%	3.98	4.01	0.8%
1	4	7/17/04	15	14.97	0.2%	3.98	3.96	0.5%
1	4	8/24/04	13.31	14.49	8.5%	3.77	3.79	0.5%
1	5	7/17/04	13.27	13.26	0.1%	3.46	3.46	0.0%
1	5	8/24/04	12.76	12.26	4.0%	3.33	3.34	0.3%
2	6	7/17/04	11.29	11.32	0.3%	4.03	4.05	0.5%
2	6	8/17/04	10.62	10.25	3.5%	4.13	4.12	0.2%
2	7	7/17/04	10.81	12.1	11.3%	5.93	5.84	1.5%
2	7	8/17/04	9.84	10.67	8.1%	5.59	5.7	1.9%
2	8	7/17/04	11.94	12.01	0.6%	5.92	5.64	4.8%
2	8	8/17/04	10.85	11.62	6.9%	5.69	5.87	3.1%
2	9	7/17/04	14.55	13.71	5.9%	5.15	5.08	1.4%
2	9	8/17/04	14.1	13.97	0.9%	5.34	5.34	0.0%
2	10	7/17/04	13.89	13.72	1.2%	3.99	3.92	1.8%
2	10	8/17/04	13.21	13.52	2.3%	3.83	3.75	2.1%
3	11	7/15/04	12.52	12.37	1.2%	4.49	4.55	1.3%
3	11	8/18/04	12.79	12.75	0.3%	4.3	4.35	1.2%
3	12	7/15/04	12.42	12.86	3.5%	3.35	3.27	2.4%
3	12	8/18/04	12.68	12.52	1.3%	3.36	3.52	4.7%
3	13	7/15/04	12.54	14.85	16.9%	2.51	2.52	0.4%
3	13	8/18/04	13	13.37	2.8%	2.64	2.67	1.1%
3	14	7/15/04	12.08	11.92	1.3%	4.06	4.12	1.5%
3	14	8/18/04	12.88	12.98	0.8%	4.07	4.1	0.7%
3	15	7/15/04	11.14	14	22.8%	4.7	4.72	0.4%
3	15	8/18/04	12	14.36	17.9%	4.95	4.68	5.6%
4	16	7/15/04	11.85	14.2	18.0%	3.41	3.37	1.2%
4	17	7/15/04	12.08	11.66	3.5%	3.02	3.01	0.3%
4	18	7/15/04	12.02	11.96	0.5%	3.55	3.52	0.8%
4	19	7/15/04	11.93	11.82	0.9%	4.4	4.51	2.5%
4	20	7/15/04	12.16	12.16	0.0%	3.6	3.57	0.8%
4	20	8/4/004	12.5	11.6	7.5%	3.96	3.89	1.8%
4	21	7/15/04	12.84	15.06	15.9%	5.37	5.16	4.0%
5	22	7/28/04	11.29	11.16	1.2%	5.36	5.15	4.0%
5	22	9/15/04	9.6	13.66	34.9%	5.59	5.56	0.5%
5	23	7/28/04	10.65	10.68	0.3%	3.14	3.13	0.3%
5	23	9/15/04	10.75	11.51	6.8%	2.96	2.89	2.4%
5	24	7/28/04	12.84	12.6	1.9%	5.17	5.12	1.0%
5	24	8/4/04	12.65	12.71	0.5%	4.81	4.84	0.6%
5	25	7/28/04	10.75	11.42	6.0%	3.76	3.73	0.8%
5	25	8/4/04	12.15	11.6	4.6%	3.96	3.89	1.8%
5	26	7/28/04	9.77	10.04	2.7%	3.25	3.31	1.8%
5	26	8/4/04	9.89	10.4	5.0%	3.37	3.31	1.8%
5	27	8/4/04	12.34	12.99	5.1%	5.12	5.14	0.4%
5	27	9/15/04	14.24	10.74	28.0%	4.27	4.28	0.2%
5	28	8/4/04	12.03	12.3	2.2%	4.18	4.14	1.0%
5	28	9/15/04	11.84	12.44	4.9%	4.15	4.14	0.2%
5	29	8/4/04	12.32	12.93	4.8%	4.59	4.7	2.4%
5	30	9/15/04	10.75	11.56	7.3%	4.04	4.05	0.2%
		average *RPD			6.0%			1.5%
		std deviation			0.075			0.014
		%CV			6%			1.6%

*RPD (relative percent difference) = 100 *(|result 1 - result 2| / average(result1, result 2))

%CV = $\sqrt{\square^2/2} \div \bar{x}$, *where* $\square$ *is for* (*result*1 $\square$ *result*2), *and* x *is for all results*

Appendix 2: Statistical Analyses with regard to assessing the predictive power of ChE data

A.2.1. Within-Person ChE Variability Estimated from Paired Data

This analysis is based on the 605 handlers with at least one periodic test observation (table A2.1.). The purpose of this analysis is to determine whether there is significant depression of cholinesterase at first periodic test as measured in plasma levels and red blood cells. The analyses for this section use the actual cholinesterase levels, not the percent depression. The within subject percent depression will be calculated from the observed within subject variability of actual levels and the average level.

A.2.2. Serum ChE Analysis

Table A2.1. Serum ChE levels at baseline and first periodic test. Change is expressed as (baseline—periodic). Percent depression as 100*(baseline—periodic)/baseline.

Measurement	Number	Mean	Standard Deviation	Minimum	Maximum
Baseline	605	4.73	0.80	2.07	6.39
First periodic test	605	4.32	0.80	1.62	6.34
Change	605	0.41	0.64	-2.00	3.67
Percent depression	605	7.96	13.96	-79.7	63.5

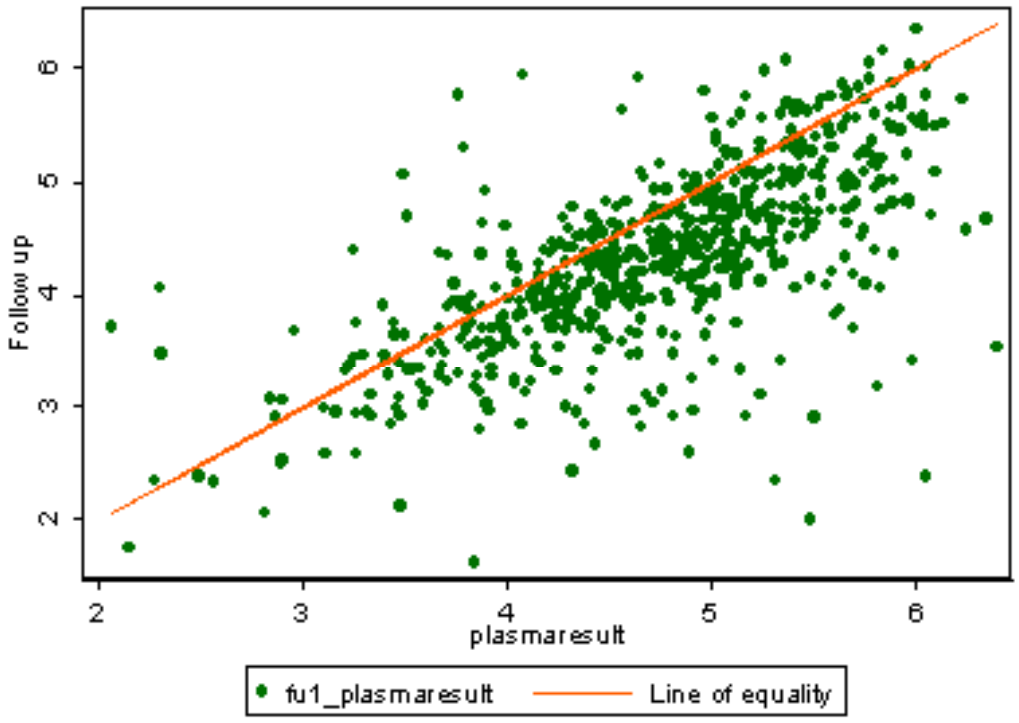


Figure A2.1. 4.4. Plot of 605 plasma measurements at baseline and first follow up. Line

represents equal values at baseline and follow up. Values above the line represent higher values at follow up as compared with baseline. Values below the line represent lower values at follow up.

The analysis of variance of plasma levels gave the following results.

Table A2.2. Analysis of variance of actual serum ChE levels at baseline and first periodic test.

Sources of Variation	Degrees of Freedom	Mean Square	F	P
Time	1	50.73225	230.4	<0.001
Subjects	604	4.09432	18.5	<0.001
Within Subjects	604	0.20213		

This analysis indicates that there is a highly significant arithmetic change in cholinesterase levels from baseline to time 1.

There was a highly significant drop in serum ChE levels from baseline to first periodic test among handlers. There was a highly significant variability in serum ChE levels among subjects. The within subject variability as a standard deviation after removing trend from baseline to first periodic test is estimated to be 0.4495. This translates to a coefficient of variation at mean baseline level of approximately, 0.4495/4.7242=0.095 or 9.5%.

A.2.3. RBC ChE Analysis
Conditions the same as in 5.2

Table A2.3. RBC ChE levels at baseline and first periodic test. Change is expressed as (baseline—periodic). Percent depression as 100*(baseline—periodic)/baseline.

Measurement	Number	Mean	Standard Deviation	Minimum	Maximum
Baseline	605	12.26	1.42	8.35	17.4
First periodic test	605	12.42	1.32	8.44	16.9
Change	605	-0.16	1.53	-4.82	5.48
Percent depression	605	-2.14	12.21	-50.2	34.3

The analysis of variance of RBC levels gave the following results.

Table A2.4. Analysis of variance of actual RBC ChE levels at baseline and first periodic test.

Source of Variation	Degrees of Freedom	Mean Square	F	P
Time	1	7.76	6.65	<0.01
Subjects	604	10.36	8.88	<0.001
Within Subjects	604	1.17		

This analysis indicates that there is a highly significant arithmetic change in cholinesterase levels from baseline to time 1.

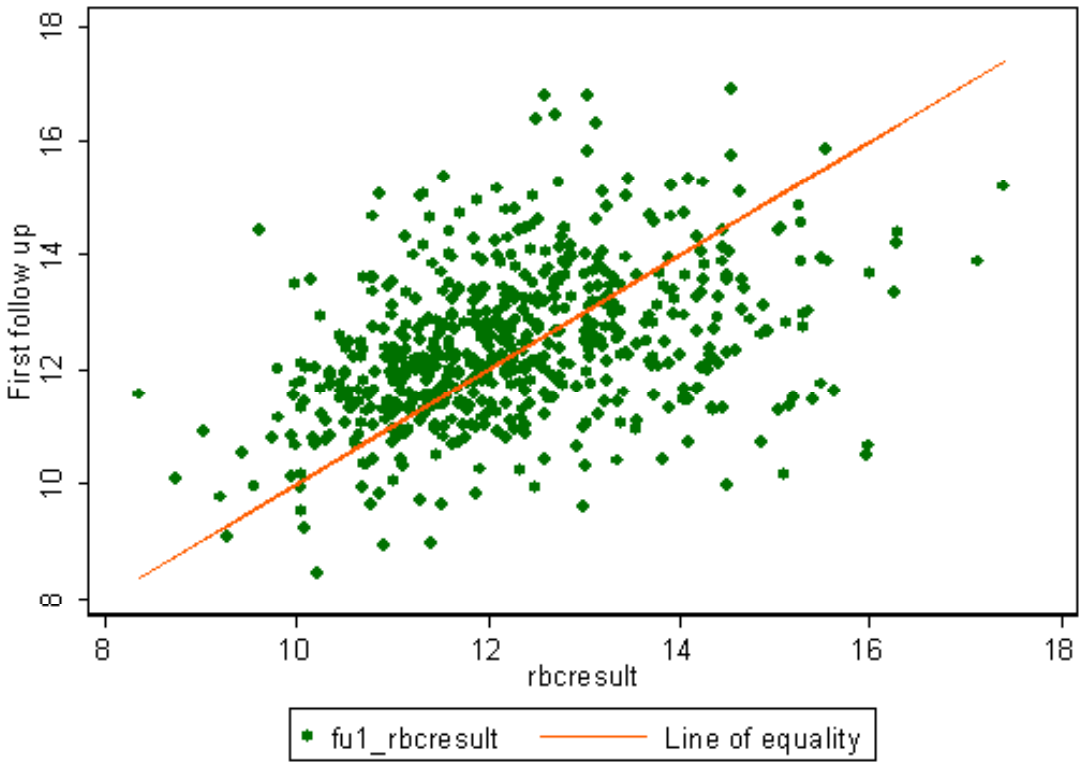


Figure A2.2. Plot of 605 RBC measurements at baseline and first follow up. Line represents equal values at baseline and follow up. Values above the line represent higher values at follow up as compared with baseline. Values below the line represent lower values at follow up.

There was a highly significant change in RBC ChE levels from baseline to first periodic test. This change represents an increase from baseline—not a decrease. There was also a highly significant variability in RBC ChE levels among subjects. The within subject variability as a standard deviation after removing trend from baseline to

first periodic test is estimated to be 1.0805. This translates to a coefficient of variation at mean baseline level of approximately, 1.0805/12.2601=8.8%

A.2.4. False positives and false negatives.

A false negative result occurs when there is real depression but the test is negative. Similarly a false positive occurs when there is no depression but the test indicates depression. This can be summarized in the following table. The likelihood of false negative or false positive results will depend on bias in monitoring data, which would increase the probability of false positives if the bias is positive (higher depression than actual), or increase the likelihood of false negatives if the bias is negative (lower depression than actual). The second factor contributing to false results is random variation in the data. Increased variability in monitoring data (which includes not only lab imprecision, but variation due to random effects from sample collection and shipment as well as variation in personal ChE activity that is not related to workplace exposures) will cause increased numbers of both false positive and false negative results.

	State of Nature	
Test Result	**Positive**	**Negative**
Positive	True positive	False positive
Negative	False negative	True negative

Table A2.5. Depiction of options for potential states of nature (characteristic present vs. characteristic not present) vs. test results.

Bias can only be assessed by comparing measured results with a known true value. There is no such comparison available for 2004 monitoring data. The effect of data variability on the number of false positive and negative results can be evaluated, and requires knowledge of: (a) the variability of the % depression data (which is treated as a coefficient of variation, "%CV"); (b) the actual value of ChE depression (either known or assumed), and (c) the levels of ChE depression used to trigger actions (referred to as "threshold levels"). In the following section, the relationship among those three factors in determining the expected number of false positive or negative factors is illustrated for hypothetical cases, and then will subsequently be applied to actual 2004 monitoring data.

The ChE monitoring rule requirements are expressed as percentage depression of plasma and RBC ChE levels. However, the application of a percent depression threshold for classifying results is intended to ask the question "do these results indicate significant exposure?" (at two levels of significance corresponding to the actions triggered). The question implied by applying (for example) a 20% threshold is NOT "does this person have exposure sufficient to depress ChE by 20%?". If that were the question, the probability of a correct result from a test would always be at 50% if there is no bias in the measurement, because with any measurement process, a normal distribution of values around a 20% mean will place half of the values below 20%. (This would likewise be the case for any action level selected, and for any level of data precision.)

A.2.4.1. False Positive rates based on variability and on the threshold value

The likelihood or expected frequency of false positive results using hypothetical threshold values of 20%, 30% and 40% is calculable using the within-person coefficient of variation, and assuming no actual exposure (that is, the natural variability of levels within handlers). From sections A.2.2.and A.2.3., this variability in actual monitoring data is estimated to be around 10% for plasma and 9% for RBC. For convenience we will assume 10% as a reasonable value. The sensitivity of this assumption will be tested by comparing all results with coefficients of variation within persons ranging from 5% and 15% percent.

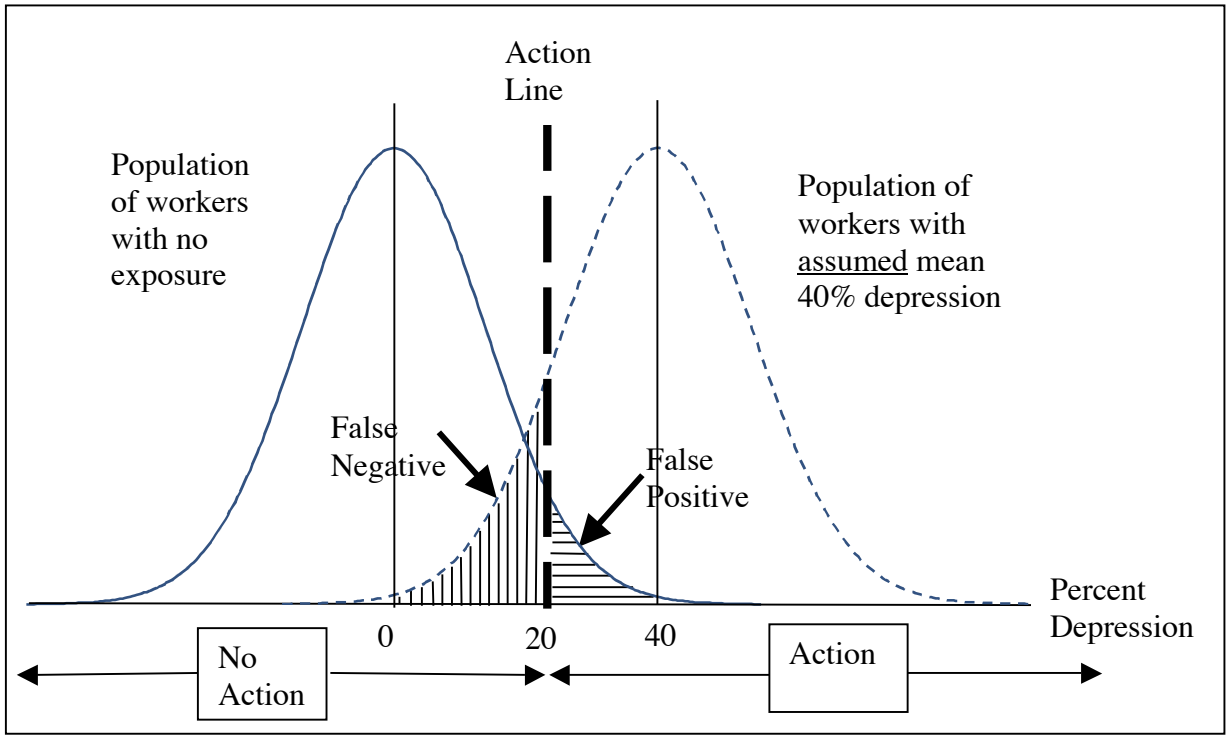


Figure A2.3. For a given true value and degree of variation (10% CV in this illustration) in measured results, a fraction of all results will fall above a threshold value. The area with the horizontal cross-hatching indicates the fraction of all values giving a false positive result for a threshold value of 20%, under the assumed amount of variability. The cross hatched area with vertical crosshatching is the fraction of results for workers with 40% true depression giving false negative results for a threshold value of 20%.

We can calculate the probability of apparent but false depressions as a function of the estimated variability for those having zero actual depression. (Of course, persons with actual depressions of, for example, 5% will not contribute to false positive results because they are truly exposed. The fraction of samples tested that come from handlers having zero actual exposure will contribute a number of apparent depression cases, based on the variability of monitoring results; the number of such false positives depends on the size of the group having no exposure but the maximum value is if all workers tested have zero exposure). The following table presents the results for such calculations.

Given % Coefficient of Variation and assuming no bias and zero exposure, what is the probability of a false positive result?

	action level		
%CV	20%	30%	40%
5	0.08%	< 0.01%	< 0.01%
6	0.43%	< 0.01%	< 0.01%
7	1.21%	0.02%	< 0.01%
8	2.43%	0.08%	< 0.01%
9	3.98%	0.25%	< 0.01%
10	5.73%	0.58%	0.02%
15	14.64%	4.63%	0.80%

Table A2.6.: Probability of False Positive results as a function of %CV and action level

For example, if the within-person random variability is 10%, then the probability of observing by chance in an unexposed worker a depression of 20% is about 0.057, or 5.7%. The probability of observing a depression of 30% by chance is about 0.58%. By the usual statistical standards, this low level of probability would be considered evidence for rejecting random chance as the explanation for a single result showing this much depression. That is, one would conclude that there is real depression. From a public health point of view it can be debated whether the level should be set that stringently. A better cut off level could be a probability of 0.20. Using this criterion, even with a coefficient of variation of 15% it would be unlikely that a depression of 20% occurred by chance alone in a given instance. At the same time, even low probabilities will produce false positive cases if enough tests are run. Testing 1000 individuals who have no true exposure would be predicted to yield 6 apparent (false positive) cases of depression at the 30% level, under these assumptions.

A.2.4.2. False negatives

To calculate a false negative we have to estimate the likelihood of a pesticide handler who has depression of cholinesterase levels testing negative according to various threshold levels. Since there is no way to know who is truly exposed or what levels of depression are actual, other than by relying on test results, we can only consider this hypothetically. In order to do these calculations we have to assume some underlying level of depression. For purposes of this example we assume that the true depression levels are 20%, 30%, and 40%. The likelihood of a false negative result is then a function of the variability around the true value, the true value itself and the cutoff for recognizing ChE depression (20%, 30%, 40%). As can be seen from the illustration above, the greater the difference between the true value of depression and the value of the threshold for recognizing depression, the fewer will be the number of false negative results. If the true level of depression is 40%, there will be fewer false negatives with a cutoff of 10% than a cutoff of 20%. One way to consider this is to ask: " what level of

true depression would it take to have a 95% chance of recognizing depression (assuming a given level of variability in the monitoring data)?".

Given %CV and assuming zero bias, what level of actual ChE depression would give a **95**% chance of triggering an action?			
	action level		
%CV	20%	30%	40%
6	30.4	39.1	47.8
7	32.0	40.5	49.0
8	33.6	41.9	50.2
9	35.1	43.2	51.3
10	36.6	44.5	52.5
Given %CV and assuming zero bias, what level of actual ChE depression would give a **75%** chance of triggering an action?			
	action level		
%CV	20%	30%	40%
6	24.5	33.9	43.3
7	25.1	34.5	43.9
8	25.9	35.1	44.4
9	26.6	35.8	44.9
10	27.3	36.4	45.5

Table A2.7. – True Exposures and False Negative Predictions

The tables above illustrate this. For the range of %CV implied by 2004 monitoring data, the likelihood of missing a positive case does not drop to 5% or less until the action level was lower than actual depressions by 10 to 15 %. depression. For 25% false negatives or fewer, action levels at least 5-7 % depression below the actual level would be required. Another useful observation is that the probability of a false positive (for unexposed workers) is less than the probability of a false negative (assuming zero bias) for individuals having true exposures at or below twice the threshold or action limit.

Calculation approach: This calculation is based on that requirement and the implied statistical fact that the cholinesterase levels are inherently log-normally distributed. This assumption can be tested and will be examined in a subsequent report.

The coefficient of variation in the arithmetic scale is approximately the standard deviation in the logarithmic scale (see for example, van Belle, G. (2002), *Statistical Rules of Thumb,* Wiley, New York, Section 5.2). In addition percentage depression can be translated to a logarithmic scale by considering that,

$$\frac{\text{Baseline - Follow Up}}{\text{Baseline}} = 1 - \frac{\text{Baseline}}{\text{Follow Up}}$$

Or,

$$\frac{\text{Baseline}}{\text{Follow Up}} = 1 - \frac{\text{Baseline - Follow Up}}{\text{Baseline}}.$$

Let

$$y = \log_e \frac{\text{Baseline}}{\text{Follow Up}} = \log_e(\text{Baseline}) - \log_e(\text{FollowUp}).$$

Then the variance within subjects on the logarithmic scale is approximated by twice the coefficient of variation(CV) squared. That is,

$$\text{var}(y) = 2.CV^2.$$

4.2 “For what true depression rate would a cut off value of 20%(30%, 40%) generate a 5% false negative rate.”

Figure A2.4. False negative rates for 20%, 30%, 40% recognition levels.

The bottom red horizontal line is at n=50. This corresponds to a 5% false negative rate. To achieve a 5% false negative rate at the 20% cut off level requires a true level of depression of 37.5% or more. Using the 30% criteria requires a true level of depression of about 45 % or more. For the 40% rule the value would have to be greater than 50%.

4.3 Derivation of false negative values.

The trick is to consider reduction rather than depression. That is, let B and F be baseline and final values.

Then,

$$\frac{B - F}{B} = 1 - \frac{F}{B}$$

So that

$$\frac{F}{B} = 1 - \frac{B - F}{B}$$

For example, a 20% depression in ChE corresponds to a proportionate reduction of 0.2 and the reduction in baseline is 0.8 or 80%.

Let

$$y = \frac{F}{B}.$$

Then we know that $\log_e(y)$ is approximately normal with mean=true reduction=□, and variance $2CV^2$. To generate the false negative curve we then take a reasonable range of true depression values from, say no depression to 50% depression. This corresponds to a reduction in y from 1 to 0.5.
We calculate,

$$z = \frac{\log_e(\text{Criterion reduction}) - \log_e(\square)}{\sqrt{2}CV}$$

For a range of true reductions from 1 to 0.5. We then calculate the area under the normal curve from z and translate the reduction to a depression. The following illustrates these calculations. The figure below (A2.4.) is based on calculating the quantities for 1000 values.

Example:
True depression = 30%
Criterion is 20% cut off

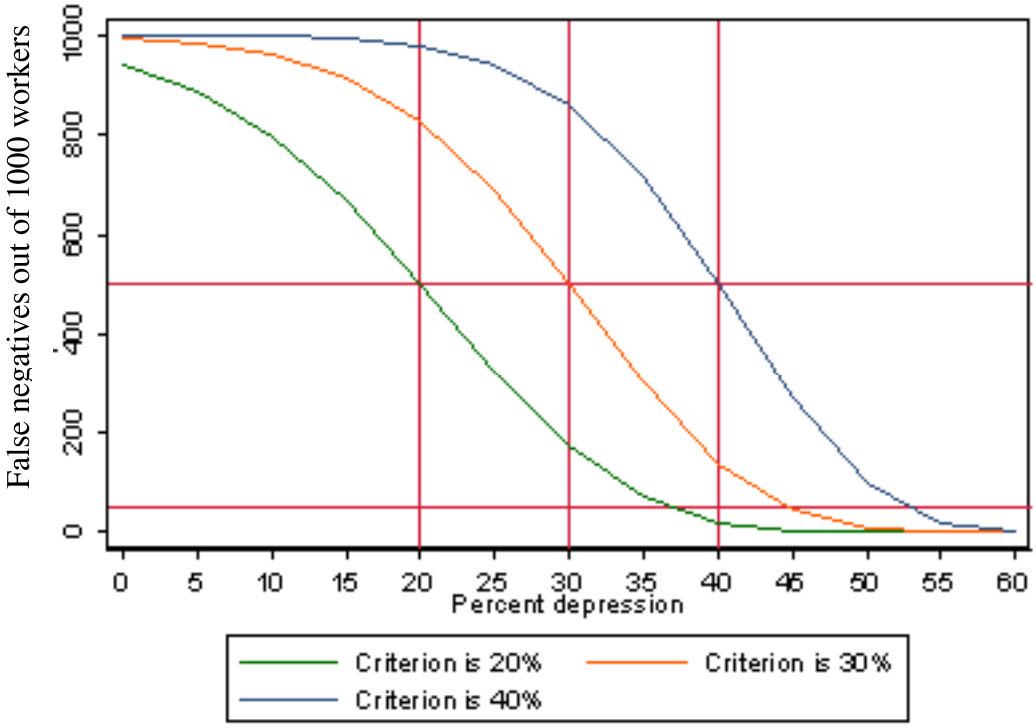
Calculations:
Reduction is to 70%
Criterion reduction is to 80%

$$z = \frac{\log_e(0.80) - \log_e(0.70)}{(\sqrt{2}) \times 0.1} = 0.9442096$$

P(z>0.9442096)=0.1725313.
n=1000*0.1725313=172.5313 is the number of positives who will be misclassified as negative.

This corresponds to the bolded line in the printout on the next page. The graph is a plot of number of misclassified workers vs. true depression level for each of the three criterion levels. For example, for a probability of misclassification (false negative) or 0.05 with Criterion =20% the true depression has to be somewhere between 35% and 40%. For the 30% criterion a little bit less than 45% and for the 40% criterion between 50% and 55%.

Figure A2.4.: False negative rates at three ChE depression recognition levels based on actual ChE depression



A.2.5. Application to 2004 Monitoring Data

From the analysis of 2004 monitoring data, there is some indication of bias leading (on a population basis) to lower than actual amounts of depression, notably for RBC ChE results. This will tend to add to the number of cases of false negatives that would be predicted from considering random error alone. The magnitude of the bias can not be determined from existing 2004 data, but a minimum estimate might about -2 %depression (based on the analysis in section 1 and assuming zero actual depression).

It was also estimated that the variation in within-person ChE changes was about 9%CV for RBC ChE depression and slightly lower than 10%CV for serum ChE depression. The framework for applying this finding to the analysis of positive and negative error presented above is shown in the following figure (A2.5.). All of the cases classified by the test as positives ("apparently exposed") are either True Positives or False Positives ("positive" here refers to the test result, not to the true state). Likewise, those cases not exceeding the depression cutoff are either actually unexposed (true negatives) or actually exposed (false negatives). The total number of exposed people is the sum of TP and FN; the total number of actually unexposed people is TN + FP. The values shown in bold in the figure below are obtained from the monitoring data; the values in italics are simple differences. For the other quantities, only one value may vary: all the rest will be determined as a result. By statistical definition, FP is calculable from A^- and the false positive rate developed in the previous discussion (0.0573 at 20%

depression, 0.0058 at 30% depression, 0.0002 at 40% depression): FP = [false positive rate * (A^-)].

Figure A2.5. True Positive-True Negatives and exposure status.

Test	True State: Positive	True State: Negative	Test results
Above cutoff	True Positives (TP)	False Positives (FP)	**Apparently exposed cases (=TP + FP)**
Below or equal to cutoff	False Negatives (FN)	True Negatives (TN)	*Apparently unexposed cases (=FN + TN)*
	Actual number of true positives (A^+), = TP+FN	Actual number of true negatives (A^-), = FP + TN	**Total number of cases**

The number FN, for example, can be as low as 0 or as high as all of the apparently unexposed cases (calculated from number of cases and number of "hits"). In the latter extreme, TN = 0 and the number of FP = (A^-). This can only be true and satisfy the definition of FP as [(A^-) * false positive rate] if both FP and A^- are 0. This case predicts maximum numbers of exposed people, of whom only a small fraction are recognized by the test.

In the former case with FN=0, TN is equal to all of the apparently unexposed cases. FP is therefore calculated to be (fp rate * A^-), where A^- is algebraically equivalent to TN / 1-(fp rate), and in the special case where FN = 0, TN is equal to all apparently unexposed cases. TP is now determined by simple subtraction of FP from all apparently exposed cases.

Applying these relationships to the Year 1 ChE depression data yields the result shown in Table A2.8., which accounts for the effect of random variation but not for bias in the data. For 2004, a total of 911 cases of periodic testing (with associated baseline values) were considered. This number is fewer than the total data set because of exclusion of follow-up tests (where ChE depression to the exposure-removal level has been observed), non-covered workers, and samples taken at shorter intervals than 1 month[1]. These analyses indicate that there is a high probability of cases of true exposure, that these are more readily discerned from the serum ChE data than from the RBC data, and that at the exposure removal thresholds (30%, 40% depression), the reliability of the tests is 87% and 99% for RBC and serum ChE tests, respectively. Even at the noisier 20% work practices alert level, a positive serum ChE test is correct better than 4 times out of 5. (It should be stressed that the unit of analysis here (a "case") is a blood sample, not a person. For estimates of frequency of occurrence of depression in people, see section 4 of this report.)

[1] This data set was selected to match the dataset reported by L&I (footnote 2) as covering all periodic tests from workers covered by the rule.

RBC ChE depression	>20% depression	>30% depression	> 40% depression
False Positive Rate	3.98%	0.25%	0.003%
Number periodic tests	911	911	911
**observed positives	47	15	3
*upper bound number, false positives	≤ 35.80	≤ 2.28	≤ .03
*% of positives that are false (upper limit)	≤ 76%	≤ 15%	≤ 1%
*lower bound number, true positives	≥ 11.2	≥ 12.7	≥ 3.0
* % of positives that are true (lower limit)	≥ 23.8%	≥ 84.8%	≥ 99.1%
* least % of all tests that are true positive	≥ 1.2%	≥ 1.4%	≥ 0.3%
Serum ChE depression	>20% depression	>30% depression	> 40% depression
False Positive Rate	5.73%	0.58%	0.02%
Number periodic tests	911	911	911
**observed positives	155	64	29
*upper bound number, false positives	≤ 31.32	≤ 2.15	≤ .03
*% of positives that are false (upper limit)	≤ 20%	≤ 3%	≤ .09%
*lower bound number, true positives	≥ 123.68	≥ 61.85	≥ 28.97
* % of positives that are true (lower limit)	≥ 80%	≥ 97%	99.9%
* least % of all tests that are true positive	≥ 13.6%	≥ 6.8%	≥ 3.2%

* This limiting value assumes NO cases of false negative tests.
** These numbers of observed positive cases are not the same as cases at "work practice alert level" or "exposure removal level". These are all tests exceeding the action level threshold. (For example, the 29 cases > 20% RBC ChE depression are made up of 19 tests greater than 20% depression but < 30%, 9 tests ≥30% depression but < 40%, and 1 case >40%).

Table A2.8.: Estimates of True and False Positives in 2004 Pesticide Handlers' data

Moving beyond this limiting case, the number of FP and FN will vary as a function of the amount of actual (versus detected) exposure. The number of recognized cases is fixed according to the numbers seen in Year 1 monitoring. If the minimum prevalence of exposure (20% cutoff) is around 10%, and the actual prevalence increases above that, the rate of FP and FN will vary as shown in Figure A2.6.:

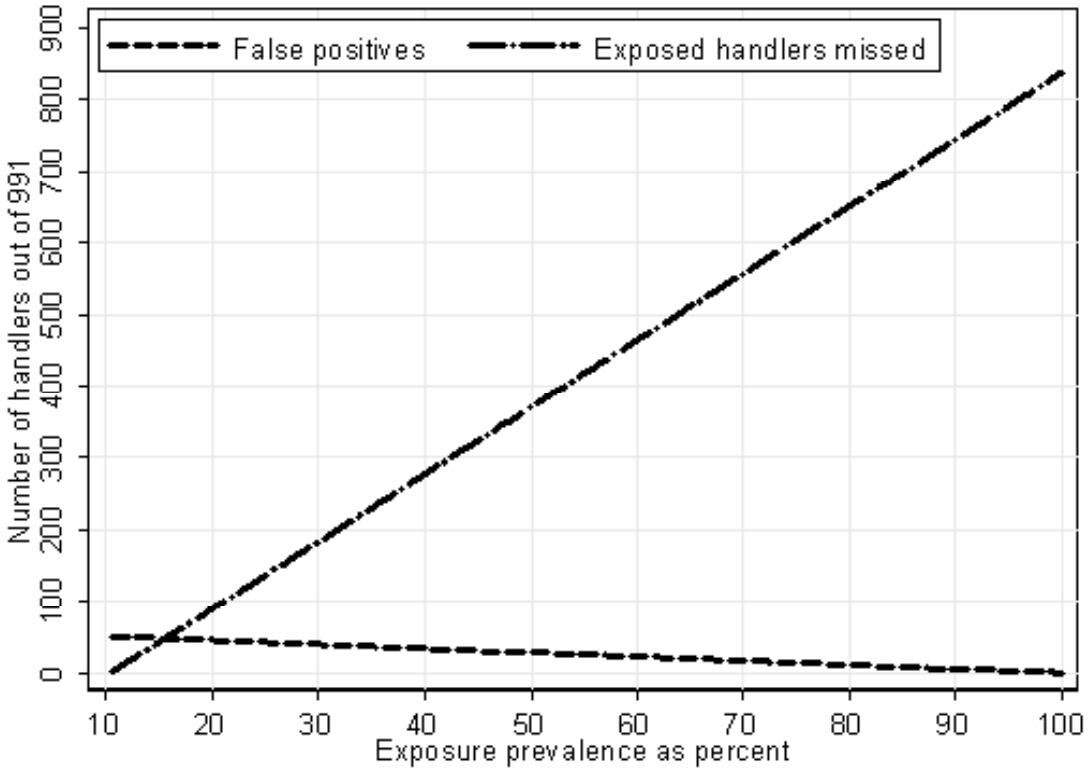


Figure A2.6.: . False positive and false negative rates for observed data on 991 periodic tests, 154 of which showed depressions greater than 20%. A false positive represents a handler incorrectly classified as exposed; a false negative represents an exposed pesticide handler incorrectly classified as not exposed. Exposure for this example means a depression of 20% or more in Serum ChE.

This figure illustrates that prevalence of exposure affects the false negative rate more than the false positive rate. This is to be expected since the false positive rate is fixed at 0.0573 according to the analysis of variance for Year 1 Serum ChE data. See the text for an illustrative calculation**.**

A similar figure can be constructed for the use of a 30% cut off point.

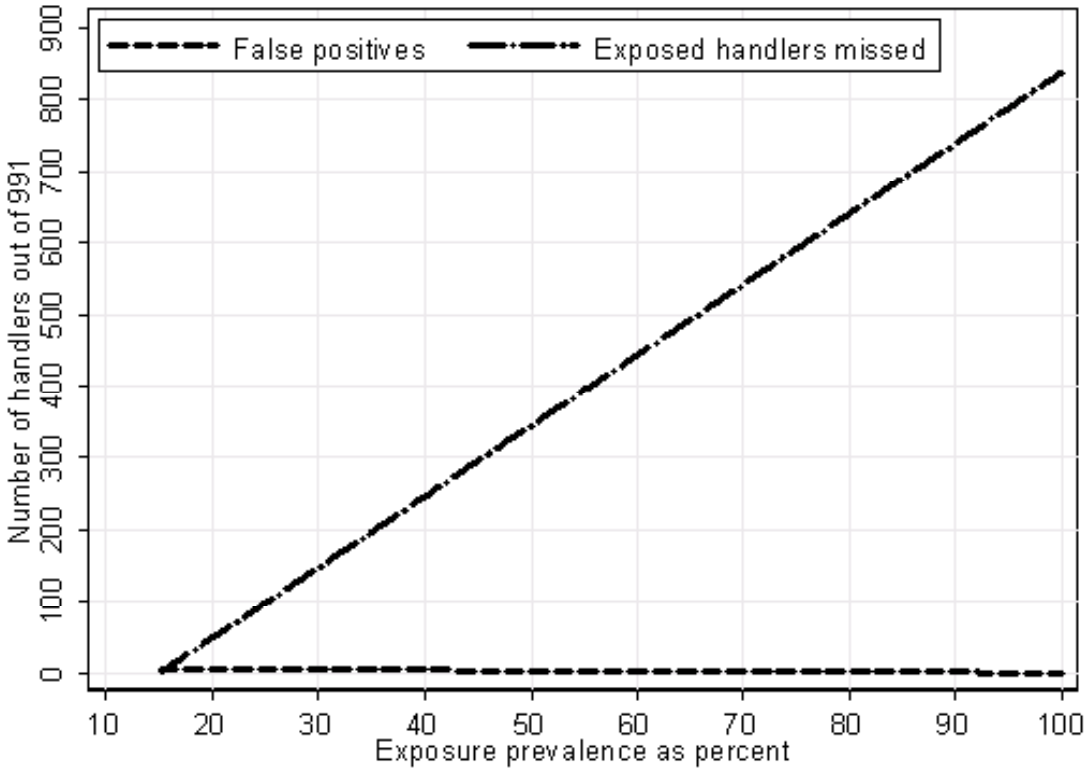


Figure A2.7. Similar to previous figure but now based on a cut off of 30% depression. Again, the false negative rate dominates.

Example calculation for plasma data.

Starting information:
1. Total pesticide handlers with at least one follow up is 991
2. 154 pesticide handlers with depression greater than 20%
3. 837 pesticide handlers with depression less than 20%
4. False positive rate at this cut off was calculated to be 0.0573.

Calculations
It turns out that once we specify the prevalence of true exposure then the whole table becomes calculable.

Specifically, suppose the actual prevalence of exposure is 20%. That implies that,

$$A^{+}=0.30*991=198.2$$

Then,

$$A^{-}=998-198.2=792.8.$$

We assume that the false positive rate is 0.0573, based on the previous calculations.
Hence the expected number of false positives among the 792.8 handlers, is

$$FP=0.0573*792.9=45.43.$$

This fixes the number of true positives, since TP+FP=154 (by assumption).

Hence,

$$TP=154-45.43=108.57.$$

Finally, we calculate the value for FN by the fact that $TP+FN=A^{+}=198.2$ so that

$FN=198.2-108.57=89.63.$

These can then be summarized in the table,

Table A2.9. Expected frequencies out of 991[2] assuming an exposure prevalence rate of 20%

Test	True State		Test
	Positive	Negative	results
>20%	TP=108.57	FP=45.43	154
≤20%	FN=89.63	TN=747.37	837
	A^{+}=198.2	A^{-}=792.8	991

The values of 89.63 and 45.43 are plotted against the assumed prevalence of 20%.

[2] This was the total number of periodic tests classified as pesticide handlers as of the time this analysis was done. The number has subsequently been corrected to 954.

Appendix 3: Stakeholder Questions and Science Advisory Committee Responses.

Page number references refer to the Draft 2004 Report.

1.13 Comment: *13.22 How were the various responsibilities and accountabilities listed in the chapter monitored for compliance? Was compliance routinely monitored for industry, health care providers, and regulatory agencies or just selected groups?*

Reply: The roles and responsibilities listed are derived from three sources; the WAC 296-307, the Guidelines for Health Care Providers or the PHL's Standard Operating Procedure. WRD 33.27 describes compliance protocols. WISHA Policy & Technical Services (P&TS) may refer a case of cholinesterase depression to field enforcement staff when either: 1) a timely consultation cannot be scheduled with the employer, 2) the employer has cases of multiple or repeated depressions, or 3) if an employer does not agree to and schedule a consultation in a timely manner. The focus of the inspection will be the employer's cholinesterase monitoring program and their compliance with the worker protection standard. In addition, worker complaints and referrals from other agencies are scheduled following existing WISHA guidelines.

Many of the roles and responsibilities were clearly achieved (e.g., Department of Health developed a laboratory requisition slip, L&I prepared and distributed provider guidelines) in the absence of formal monitoring. Other processes were discussed in the bi-weekly meeting of a DOH and L&I working group or by Scientific Advisory Committee (e.g., timeliness of the lab mailing results to providers and timeliness of DOH reporting depressions to L&I). Many of these processes were found to work smoothly and others were found to need improvement. For those activities that needed improvement and were responsibilities of DOH or L&I, the agencies made appropriate changes where feasible within resource constraints. Other responsibilities and accountabilities were not monitored due primarily to resource constraints. The Scientific Advisory Committee noted several areas where the program would have benefited from more systematic monitoring (e.g., developing a better understanding of how providers communicated with employers and pesticide handlers in the program). The provider survey at the end of the program attempted to address some of the provider issues (See item 3.03). Most of the monitoring focused on DOH and L&I to assure that their processes were running smoothly. This was likely due to the need for high quality laboratory data and that DOH and L&I had most ability to fix problems that were identified within their own agencies.

1.15 Comment: *Information should make clear the numbers and percentages of workers with significant depressions. There was a lot of confusion this year regarding what the correct "denominator" was for calculating the percentages of workers (as opposed to follow-up tests) with significant depressions. L&I need to get a better handle on these numbers.*

Reply: We concur and are discussing with L & I how to accomplish this.

1.19 Comment: *Pg. 28.5 Is the statistic of 93% of pesticide applicators/handlers Hispanic really representative of the overall population? How can this be verified*?

Reply: What overall population is being referenced? We presume that the population of workers covered by the Rule is well represented in this group. It is possible that Labor and Industries has more demographic data on pesticide handlers in the state, but we did not consider this.

2.01 Comment: *Another item discussed by Stefan and Matt at the meeting was an employer that didn't report handling but had no exposure. This employer is in the database as two employers, 17 and 389. 29 employees received baseline tests (employer 17) and one periodic test (employer 389) but they had no exposure. The employer claims not to use covered pesticides and provides the tests under their union contract. L&I contacted the employer to obtain handler hours reports and was told the employer didn't think they needed to report because they didn't have exposure.*

Reply: The 2004 Report from the Scientific Advisory Group indicates the need for better information and outreach between L & I and employers in order to improve accuracy and response rates for reporting of worker hours. Clarification of the requirements under the Rule for reporting of hour is part of this response.

2.03 Comment: *More Data Needed There were significant gaps in the information necessary for a complete analysis of the implementation of the rule. We are all in agreement that there needs to be a more thorough compilation of data including, but not limited to, the following: handling hours for every employee that was tested; time between notification of removal and actual removal; full name of pesticides used; description of engineering controls/closed systems/all PPE used; interviews of all employees with depressions to the alert or removal level; and interviews of a representative sample of employees who declined testing.*

Reply: This view is consistent with the recommendation of the 2004 Report from the Scientific Advisory Group.

3.03 Comment: *Health care providers are directed to collect a pre-exposure history and to review differential diagnosis to determine whether a pre-existing condition may be causing the depression. Furthermore, health care providers failed to attempt to identify if there were non work-related conditions that may be causing the depression.*

Reply: Health care providers were provided educational materials regarding the cholinesterase monitoring program. The contents of the 'Cholinesterase Monitoring for Agricultural Pesticide Handlers; Guidelines for Health Care Providers in Washington State' discusses the evaluation of workers with depressed cholinesterase levels for non-occupational exposures and whether a non-work related condition caused the cholinesterase depression. From the provider survey, only three of the eighteen respondents evaluated workers for non-occupational causes of cholinesterase depression. There is no formal requirement for physicians to evaluate a cholinesterase depression in every case. If there are no reasons to believe that the ChE depression was caused or effected by something other than pesticide exposure on initial history or medical evaluation, there would be no need to inquire further for a non-occupational cause for cholinesterase depression. Health care providers gave responses that additional training in the areas of 'making recommendations based on test results' is desired.
WAC 296-307-14815 requires the employer to identify a physician or other licensed health care provider who will 'interpret tests' and 'provide guidance on medical monitoring'. No evaluation of the factors considered in the employers' selection of physicians has thus far been considered by the scientific advisory committee. Chapter 5 contains recommendations related to these comments from the Scientific Advisory Committee. Pg 67.7 – 10, 67.18-19.

3.04 Comment: *Relationship with Medical Provider: Medical providers should obtain occupational history as part of the pre-exposure medical history and evaluate alternate causes in the event a depression is reported.*

Reply: See above. Also, a sample medical history form in both English and Spanish is posted on the cholinesterase providers' web page.

3.05 Comment*: Page 32 mentions non-pesticidal causes for depression. This avenue needs to be explored in greater detail with health care providers to gain the most benefit for the overall health of the employees.*

Reply: This view is consistent with the recommendation of the 2004 Report from the Scientific Advisory Group.

3.06 Comment: *Pg 4.29 symptom of constricted pupil is missing but is a significant symptom for field recognition of a problem.*

Reply: This has been added to the final report from the scientific advisory committee.

4.01 Comment: *Public Health Lab and Data Validity. The Public Health Lab (PHL) has some problems at the beginning of the year, largely due to high number of unexpected baselines because testing of large numbers of workers who evidently were not covered by the rule. Still the PHL was able to test all of the samples and generally provided the results from follow-up tests in a timely fashion. The data received by the Scientific Team, regarding the samples run by the PHL, are now the most comprehensive statistically viable data ever produced in relation with a cholinesterase medical monitoring program. The work of the Public Health Lab and the Department of Health should be commended for the consistent high quality of their work.*

Reply: we agree.

4.02 Comment: *Page 14 discusses sources of error but does not explain if, or how, the overall dataset was handled to adjust for error. We know that employees had blood drawn at different clinics. This may have affected reliability of the data. Were differences in clinics taken into account in the analysis?*

Reply: For 2004, the Scientific Advisory Committee did not examine the statistical relationship between provider and depressions. Anecdotal information from the experience with QC samples and discussions with the lab indicates that methods of collection and handling of samples could contribute to overall variability, and that there is a range of proficiency among providers in terms of successfully following the protocol fro collection and shipment of samples. This will be an item for ongoing follow-up in 2005.

4.03 Comment: *The Agricultural Stakeholder Advisory Committee was informed that the Washington State Department of Health Laboratory (PH Lab) was using scientifically recognized Standard Operating Procedures (SOP) to ensure quality control, the integrity of specimen collection, shipment, handling, storage, timing of analysis etc., however, the draft report mentions that the SOP's were changed in March. And that other overall measurements*

programs were added since January (page 15). What changes were made and why?

Reply: The overall protocol is in line with other methods in use or reported in the scientific literature, but details may need to vary depending on the specific instrumentation used. The major protocol change in 2004 was to provide additional dilution for serum samples, to reduce sample viscousity. Samples with high lipid or protein content would occasionally fail to transfer properly and cause the autoanalyzer to detect an error and shut down. While this did not result in erronius data, it did affect lab capacity and throughput. Another change was a more complete implementation of in-lab QC samples that was phased in after the start of the program. It is to be expected that the lab will cointinue to seek improvements in its detailed practices and procedures, and that each of these will need to be documented and tested for any resulting shift in analytical results.

4.04 Comment*: Page 15 states that the SOPs were developed in January, modified in March and adopted in August. This creates the perception of data quality issues, especially for data obtained during the early months. What changes were made between January and March? Why were they made? Were the data collected during that timeframe adjusted to reflect potential variation?*

Reply: these details have been added to the final version of the report. See also answer to question 4.03.

4.05 Comment: *Page 15 lists several approximations incorporated into the current program that could effect the predictive power of the assay results. 1. Use of a single enzyme substrate, 2. Use of an automated "turn-key" instrumental analyzer, etc., 3. Current method of conversion of activity to a hemoglobin basis, 4. Exclusion of samples with evident hemolysis (disruption of red cells), and 5. Use of hemolyzed but not solubilized RBC samples for analysis. Since addressing these issues would decrease sample variability and bias, why were they not included in the recommendations section of the report for potential action?*

Reply: These items were addressed in the general recommendation in the final report that both specific and general changes to the method be considered. The SAC is not in a position to propose detailed changes for every recognized question of lab procedure, but does recommend that the lab investigate these and other possible factors that might contribute to measurement error.

4.07 Comment: *Pages 15 and 16 identify several issues for further discussion with the lab and possible corrective action to reduce variability and bias, yet the recommendations at the end of the report fail to mention these items.*

Reply: the recommendations section added as Chapter 6 of the final report includes these points.

4.08 Comment: *16.26-31 Indicates the program was initiated prior to being ready. This may indicate suspect results and conclusions may have been made during the 2004 pilot program.*

Reply: It is likely that data quality will improve as the lab gains experience with this analysis and refines its methods. The 2004 data are of a known quality and data uncertainties were

considered in reaching (or declining to reach) conclusions.

4.09 Comment: *Pgs 16-17 Indicate inadequate adherence to sample quality, integrity, and analytical timeliness. If the samples are not analyzed quickly and results conveyed to the providers, employers, and handlers of what value are they?*

Reply: Improving timely communication among all of the component groups in this program is a key recommendation of this committee.

4.10 Comment: *Page 17 states that samples were accepted even when protocols were not followed. This could lead to even greater variability in the data. Nonconforming samples should have been excluded. Recommendations for stricter adherence to both sample integrity protocols and the SOP should be included in the final recommendations.*

Reply: Both of these recommendations are included in the final report.

4.12 Comment: refer back to 4.08 *The scientific draft report mentions that PHL did accept and analyze blood samples that did not meet the adopted Standard Operating Procedures. "Acceptance of questionable samples was perhaps the areas where the SOP was least rigorously applied." Page 17 refers to the fact that at least one blood sample that should have been rejected according to the SOP was accepted and run. Is the Scientific Committee confident that the test result data and your determination of the number of false negatives and false positives is accurate despite the fact that an unknown amount of the data analyzed violated the SOP, which was designed to protect the integrity and accuracy of the data?*

Reply: The calculation of false negative and positive values is an estimation that depends on some assumptions, and is a range of values rather than a precise count. The possible and observed effects of samples held beyond SPO holding times is discussed in the report.; the analysis of false negative and positive results reflects the overall data quality in 2004.

4.13 Comment: *Page 26 gives a strongly worded recommendation to the public health lab to reassess its overall methods to reduce variability and bias. These cautions need to be repeated in the final recommendations and included in any summary of the report. It also raises the concern that the lab may not have the capacity to handle an increased workload.*

Reply: this has been done, in Chapter 6 of the final report.

4.14 Comment*: How did the Scientific Committee analysis adjust to the test results taken prior to and after changes to SOP and measurement programs?*

Reply: We did not adjust or modify any of the calculated values, but we did check for the effect of storage and analysis date. The result did not identify any significant difference in the data over time, including over the period when protocol changes were implemented.

4.15 Comment*: Given the fact of the high number of false positives and negatives etc. is the Ellman colorimetric method the most accurate method for measuring ChE levels? If so, why?*

Reply: All methods will produce variability in results, which will result in false negatives and false positives. The Ellman method has the advantage that it is well developed and has been used in a comparable monitoring program already, and that off-the-shelf technology is available for its implementation.

4.16 Comment*: It was my understanding that those samples that violated the SOP would not be included in the analysis. Why weren't those samples that violated the Scientific Committee approved SOP excluded from the analysis? The recommendation on page 17 suggests excluding non-conforming results?*

Reply: The PHL did reject samples that were above its required temperature limit when received. The case of a sample appearing to have had some hemolysis was detected in a spot check; there was no systematic review to identify all such cases.

4.18 Comment: *On page 26, the committee makes a recommendation that is very important to the accuracy and reliability of the test results. However it is not highlighted nor included in the list of recommendations at the end of the report. "..the PH Lab is encouraged to reassess its overall methods in concert with experts in the field of enzymology and specifically cholinesterase enzyme characterization.…to reduce the sampling and analysis variability of ChE assays…" "The RBC assay in particular needs more refinement to reduce variability and bias to meet the needs of this monitoring program." How can the scientific committee make such statements, while at the same time appear to give the impression that the test results are scientifically relevant?*

Reply: The data as provided are useful for some analyses and to address some questions, and are of limited use or no use for other issues. There are no perfect data, and any improvements in precision and accuracy will benefit the monitoring program by reducing the numbers of false indicators and strengthening the relationships between measured ChE depression and workplace factors.

4.19 Comment: *Pg 26.31-33 What plans have been made to increase lab capacity to handle the increases in samples which will result with lowering potential handler exposure from 50 to 30 hours per 30 day period?*

Reply: information about planning and changes for the 2005 monitoring season should be obtained from Dr. John Furman at the Washington Department of Labor and Industries.

4.22 and 4.23 Comment: *The report mentions concern over the lack of a certified RBC ChE reference material. Shouldn't there be a recommendation to obtain such material, especially since the Scientific Committee report indicates that RBC test results are questionable and less reliable than the serum test?*

Reply: This need is noted as an issue in the final report

4.24 Comment: *Chapter 4 is an analysis of the Cholinesterase monitoring results, and as such, it includes a report of the L&I control measures. Please add the excel spreadsheet which*

documents the L&I Control as an exhibit to this section.

Reply: Refer to Appendix 1 of the final report..

4.25 Comment: *The L&I Control was an excellent first start but needs further work in the upcoming year. Please provide an explanation of how the L&I quality control program worked- what the program was designed to test, and how it accomplished this. The report should mention that the L&I quality control program started in mid-July, after it was authorized by the Scientific Advisory Committee. A goal next year should be to start the QC program in February.*

Reply: The results of this activity and their interpretation are found on pg 20 of the draft report; recommendations regarding continuation of the blind QC program are in the final report

---

4.26 Comment*: The L&I Control spreadsheet lists 30 participants. The draft report lists 54 baselines. Apparently, when participants returned for a follow up test, it was recorded as a second baseline. This is misleading in terms of the overall program, since we are accustomed to thinking in terms of a baseline and a follow up taken some time later. It should be explained.*

Reply: There were 30 initial sample duplicates and 24 subsequent ones. Since there was no actual exposure, the chronological order is arbitrary.

4.27 Comment: *Of the 30 participants in the L&I control, it appears that all of them had blood drawn on one occasion, and 23 of them had blood drawn on a second occasion. Since there were two blood draws at each occasion, the 23 individuals who returned for a follow up test provided four samples of blood. When comparing these four samples drawn on two separate occasions, four of the 23 participants (line 7, 19, 26, and 31 on the spreadsheet) had RBC variations greater than 20%, comparing the highest value to the lowest value. This should be added to the report.*

Reply: the range from highest to lowest value does not allow predictions about overall measurement repeatability in the same way a standard deviation does. Additionally, the worst case agreement is less important than being able to assess how often such outlier results occur.

4.28 Comment: *All of the 30 participants in the L&I control provided at least two samples of blood drawn on one occasion. There is no indication whether the two samples that were drawn at the same time were tested by the lab in the same batch, or held out and tested in different batches. Please explain this. The goal in the next year should be to hold out the samples and test them in a different batch, in order to determine if there was variation in blood drawn at the same time when run in a different batch.*

Reply: The lab is blind to which samples are duplicates, so it is not possible for them to treat duplicates differently from other samples. The original goal of duplicate submissions was to look as agreement between samples run under the same conditions. Looking at the effect of different batches and different days is also useful, but can't be separated from within-batch differences until those are characterized.

4.29 Comment: *Talk more about "stacking the deck" in favor of less error in the analysis of*

samples.

Reply: The approach recommended in the final report consists of: 1. applying acceptance criteria for samples stringently; 2. reviewing and upgrading analysis methods as much as is practical. Continued use of blind QC samples and further outside review will also help manage data quality.

4.30 Comment: *Finally, we believe that the QC program should test samples drawn from the same person, within one or two days, at two or more different facilities. It did not appear that the L&I program attempted to do this.*

Reply: If the intention is to reach statistical conclusions regarding how one provider differs from another in collection methods and the effect on results, this would require a complete study design that considered how big a variation would be detectable with different numbers of samples. This is a topic that the SAC could consider in the future.

4.31 Comment*: Pg 57 Table 5.2 Actual time between blood draw and receipt by PHL is average of 1.1 days. What was range and were samples which exceeded 48 hours discarded?*

Reply: All samples were required to be processed for freezer storage within 48 hours of collection, and according to lab reports, this occurred for all but rejected samples.

4.33 Comment: *A significant number of the baseline blood samples were taken at a couple of ChE educational meetings, and in many cases the follow-up blood tests were taken at different clinics by different medical providers and/or phlebotomists. In the draft report I did not see any reference to scientific determination related to the use of multiple clinics by the same employees.* □ *& I suggested that my non-handler ChE depression of 20.4% was most likely due to the use of two clinics. Did your analysis of test result accuracy include a review of the correlation between depressions and the fact some employees submitted blood samples at different clinics?*

Reply: Not in 2004; see answers to question 4.02 & 4.30

4.34 Comment: *Unauthorized samples submitted by the Farm Bureau Throughout the Scientific report there is discussion of the unauthorized samples submitted by the Farm Bureau as though they were a planned and approved part of the implementation of the rule. We want any reference or discussion of those specific samples to be described as "unauthorized samples." There should also be clarification when discussing the data as to whether or not those samples were included in the final totals; if so, what is the impact on the total final numbers and how it would change the overall averages of depressions and work removals; and if the unauthorized samples are not included in the total that must be clarified as well, and discuss what effect that had on the program (i.e. distortion of total numbers, PHL and L&I staff time loss in investigating the situation, etc.).*

Reply: The SAC did not merge these sample results with other data, but did look at them and found them to be consistent with the L&I QC sample results. The description of the Handler data set and the various groups of extraneous samples that were initially included has been updated. Analysis of other program impacts such as cost is beyond the scope of the SAC.

4.35 Comment: *The External QC blind and split sample data submitted by non-handlers has caused significant controversy at a Stakeholder Advisory Committee meeting, etc. Page 27, Section 4.1 refers to this unofficial data. It would help if your reference to that program could include: That the unofficial blind and split sample program was developed at the request of the Farm Bureau, by Dr. Stephen Smith MD (member of the ChE Scientific committee). Scientific Committee member Dr. Alan Felsot PhD, and Agricultural Stakeholder Committee member Kirk B. Mayer submitted samples along with other grower advocates. It should be noted that, at a joint Scientific and Stakeholder Advisory Committee meeting, held prior to the decision by the scientific committee to implement a similar program, Dr. Alan Felsot mentioned that he had submitted blood samples. It should also be noted that that none of the Farm Bureau program data was included in the scientific analysis and that upon request by DOH Dr. Stephen Smith MD submitted all information regarding the program.*

Reply: It is our judgment that the Scientific Committee's report should focus on scientific aspects of the program evaluation. For that purpose, it is important to not confuse pesticide handler samples with other samples. That being accomplished, we do not feel that it serves a useful purpose for our report to dwell on these extraneous controversies.

4.36 Comment*: The Scientific Draft report mentions the FB unofficial control group, page 27. Since there was such a controversy at the Stakeholder Advisory Committee regarding this program it would be helpful if references were a little more informative, especially for those that read the report but have little Knowledge of the program.*

If you can could suggest some language. I did mention it in my questions to the SC.

Things I would like to see reflected in the paragraph are:

1. Identify Dr. Smith as a Scientific Committee member, and that he aided the FB is setting up the program.
2. Identify Dr. Felsot,as a Scientific Committee member, added to the fact that he submitted blood.
3. That Dr. Felsot, at the Joint Scientific and Stakeholder meeting held on May 10 did comment that he had submitted blood.
4.Also mention that I (a Stakeholder Advisory Committee member)submitted tests.
5. Mention that the FB unofficial blood tests started two months before the Stakeholder Advisory Committee was notified that the ScientificCommittee had decided to do a similar type program.
6. That upon request Dr. Smith did provide information related to the FB program to the DOH.
7. And finally that the Scientific Committee has not included the unofficial test information in their scientific analysis.

Reply: see response to comment 4.35.

5.03 Comment*: In an effort to reduce the significant number of test result errors, it was suggested (at the Nov 22 Stakeholder meeting) to request that the scientific committee look at the possibility and impacts of requiring both a 20% or greater depression from the baseline sample result and that the cholinesterase activity also be greater than two standard deviations than the*

mean baseline activity established through the 2004 ChE Medical Monitoring Program. (To my knowledge no other Medical Monitoring Program has the advantage of the large number of baseline tests generated this year by the Washington program. This large number of baselines may give us an opportunity to better identify exposure related ChE depressions that other programs have not had.)

It would appear that such methodology could focus efforts on handlers having a significant change in cholinesterase activity at an activity level that would more accurately (?) indicate an actual exposure related depression??

The Stakeholder Committee would be very interested in the Scientific Committee's thoughts on this issue or any other suggestions on how to improve the reliability of the data generated by the 2004 ChE Medical Monitoring Program.

Reply: Using population ranges for ChE activity is not likely to improve reliability of the test results, because the between-person variation in ChE levels is very large. Improvement in the reliability of monitoring results can be achieved by reducing within-person variation (through improvements in samples collection, shipment, and analysis).

5.13 Comment: *Pg 31.1-21 What is the biological significance of the reduction in serum ChE and increase in RBC ChE levels?*

Reply: At present, it is our assessment that the apparent average increase in RBC ChE levels is an artifact. There is no obvious biological explanation for the change. In contrast, the apparent decrease in serum ChE activity can not be explained by measurement error and is most likely indicative of ChE inhibition from exposure to pesticides.

5.14 Comment: *Pg. 32.9-23 Does the 16.6% of serum ChE depression and the 4.9% of RBC ChE depressions justify the overall costs of this monitoring. Of these ChE depressions how many are significantly detrimental to health. How many of the depressed RBC ChE levels came from handlers with depressed serum ChE (i.e., are you measuring the same population of handlers 'twice')*

Reply: There have not been any formal reports of workers with clinical symptoms of ChE depression from the 2004 season (refer to Chapter 5 for one anecdotal report of a single instance of possible symptoms). A worker who experiences ChE depression without symptoms and then recovers baseline ChE activity would not be expected to show any long-term health effects; during the time period when ChE levels are depressed, the worker might be at increased risk of health effects if additional exposures occur.

There were 13 instances of periodic tests with both RBC and serum ChE depression to the alert level, representing 10 individuals. The total number of periodic tests with alert level depression of RBC ChE or serum ChE (including those "counted twice") was 203.

5.17 Comment: *Pg 40.7-12 Indicates pesticides are good (increase) for RBC ChE levels. This is highly doubtful. Report would indicate that analyses if serum ChE is the only test which was providing warning data. Therefore, why continue with RBC ChE testing?*

Reply: (Refer also to the reply to comment 5.13). The RBC ChE assay is more difficult analytically than the serum ChE test is. The incentive to continue to use it is that the form of the enzyme found in red blood cells is a close match to the acetylchlinesterase found in nervous system tissue and may therefore be more representative of the status in those target tissues.

5.23 Comment: *There are other ways to account for natural variation and minimize the error rate. Because of the natural variability that exists within an individual in regards to cholinesterase activity, a more comprehensive hierarchy may be necessary to identify workers with relevant and biologically significant inhibitions of cholinesterase activity. Such an effort will better focus the limited resources to those workers with either/or work habits requiring modification or significant exposures requiring immediate attention.*

Reply: Natural variation in baseline ChE activity between people is the main reason for using each worker's baseline value to interpret periodic test results. Significant depression compared to baseline may be considered indicative of exposures to ChE-inhibiting agents in every worker. Given the role of ChE monitoring s a tool for identifying and correcting exposures rather than as a means of detecting illness, it is unlikely that any workers can be identified in whom ChE depression should be ignored.

5.24 Comment: *Even under the best quality control procedures, cholinesterase levels can vary 10-15% due to variability in handling. How was this taken into account in the data analysis?*

Reply: The method of estimating within-person variability used in the 2004 Report from the Scientific Advisory Group relies on the statistical power of the entire data set of baseline and first periodic test (approximately 1200 results for each form of ChE). All sources of variation that are reflected in this sample set are considered in the calculated measures of variability. That would include variations in sample collection and handling methods.

5.26 Comment: *When I report to farmers etc. on the number of depression should I exclude the RBC depressions and only mention the serum depression rates?*

Reply: Despite the overall trend of increases in RBC ChE from baseline to periodic test, nearly 50 worker tests showed RBC depression to the alert level or more. The minimum fraction of periodic tests showing this much depression was about 1.2%, and this is almost certainly an underestimate of the true occurrence rate for RBC depression. It is very likely that instances of RBC depression are occurring, and improved methods may reveal more occurrence that was seen in 2004.

5.27a Comment: *Pg 5.28 What is his/her normal level – the level of a population of an individual*?

Reply: The statement has been changed to '"until the worker's cholinesterase activity returns to within 20% of his/her baseline".

5.27b Comment*: Pg 6.19 why 15% range and why one test instead of an average of two (does a singe baseline have an adequate database to assure normal range for an individual?)*

Reply: The draft scientific advisory report states (Pg. 6.17-21) the following:
"Existing monitoring programs use baseline determinations can be made on the basis of a single test result, or by averaging two samples taken at least three days apart. The two samples should show no more than 15% difference. If more than 15% a third sample should be taken and the two closes tests averaged. The approach adopted for the current monitoring program is for a single baseline sample to be used."
The description of the existing monitoring program is that of the California cholinesterase monitoring programs recommendations to physicians, which is available at http://www.oehha.ca.gov/pesticides/pdf/docguide2002.pdf. The purpose of multiple baseline tests is to reduce the number of false positive and false negative results related to subsequent cholinesterase monitoring. The impact of estimated baseline variability related to false positives and false negatives for the Washington State program are included in this scientific advisory report (Sec 4.5.5).

6.01 Comment: *What information would the Scientific team need to evaluate the relationship of handler hours to level of ChE depression?*

Reply: In the 2005 season, we expect to get much more complete reporting of handler hours (ideally, one report of hours per periodic blood test). Additional information regarding the nature of the pesticide and formulation used, the operation of process in which the pesticide was used, and other details will also be informative and will be obtained at least for cases of alert-level depression.

7.02 Comment: *Page 56 recommends increased outreach to employers regarding who which employees should be referred for baseline testing. While we agree this would be a positive step, the text implies that growers may have taken advantage of L&I funding. That phrase should be deleted and replaced with a recognition that pest pressures and the resulting need to spray vary with the weather, population cycles and other factors. While over time, growers will be able to better estimate how many employees need to be tested, those who are conscientious about following the rule will nearly always send more employees for baselines than eventually meet the criteria.*

Reply: This has been done.

8.17 Comment: *At the November 22 Stakeholder Committee, Michael Wood (L&I) reported that the information in the Scientific Committee report on Page 36 beginning with line 21 is an error, due to a misinterpretation of differing data sets. Without more direct and comprehensive communications between all elements (agencies, consultation, committees, etc.) involved in this program, how can we be assured that other misunderstandings of data has not occurred, impacting analysis of the rule? Such misunderstandings have also occurred at the Stakeholder Committee level.*

Reply*:* The difference appears to be in information that had changed as the report was being written. Finalizing all monitoring system data and achieving a prompt summary report are conflicting goals in this situation. While the actual growing season is concluded by the late Fall, some data will not be finalized for a period that could extend for several months (field visit reports, tardy reports of worker hours, etc). The Scientific Advisory Group is anxious to avoid inconsistencies and use of outdated information. Where such inconsistencies do occur, we will try to clearly identify them and to qualify or correct any conclusions that are affected.